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(54) Title: NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

(57) Abstract: The present invention provides novel nucleic acids, novel polypeptide sequences encoded by these nucleic acids and
uses thereof.



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NOVEL NUCLEIC ACIDS AND POLYPEPTIDES

1. TECHNICAL FIELD

The present invention provides novel polynucleotides and proteins encoded by such polynucleotides, along with uses for these polynucleotides and proteins, for example in therapeutic, diagnostic and research methods.

2. BACKGROUND

Technology aimed at the discovery of protein factors (including *e.g.*, cytokines, such as lymphokines, interferons, CSFs, chemokines, and interleukins) has matured rapidly over the past decade. The now routine hybridization cloning and expression cloning techniques clone novel polynucleotides "directly" in the sense that they rely on information directly related to the discovered protein (*i.e.*, partial DNA/amino acid sequence of the protein in the case of hybridization cloning; activity of the protein in the case of expression cloning). More recent "indirect" cloning techniques such as signal sequence cloning, which isolates DNA sequences based on the presence of a now well-recognized secretory leader sequence motif, as well as various PCR-based or low stringency hybridization-based cloning techniques, have advanced the state of the art by making available large numbers of DNA/amino acid sequences for proteins that are known to have biological activity, for example, by virtue of their secreted nature in the case of leader sequence cloning, by virtue of their cell or tissue source in the case of PCR-based techniques, or by virtue of structural similarity to other genes of known biological activity.

Identified polynucleotide and polypeptide sequences have numerous applications in, for example, diagnostics, forensics, gene mapping; identification of mutations responsible for genetic disorders or other traits, to assess biodiversity, and to produce many other types of data and products dependent on DNA and amino acid sequences.

3. SUMMARY OF THE INVENTION

The compositions of the present invention include novel isolated polypeptides, novel isolated polynucleotides encoding such polypeptides, including recombinant DNA molecules, cloned genes or degenerate variants thereof, especially naturally occurring variants such as allelic variants, antisense polynucleotide molecules, and antibodies that specifically recognize one or more epitopes present on such polypeptides, as well as hybridomas producing such antibodies.

The compositions of the present invention additionally include vectors, including expression vectors, containing the polynucleotides of the invention, cells genetically engineered to contain such polynucleotides and cells genetically engineered to express such polynucleotides.

The present invention relates to a collection or library of at least one novel nucleic acid sequence assembled from expressed sequence tags (ESTs) isolated mainly by sequencing by hybridization (SBH), and in some cases, sequences obtained from one or more public databases. The invention relates also to the proteins encoded by such polynucleotides, along with therapeutic, diagnostic and research utilities for these polynucleotides and proteins. These nucleic acid sequences are designated as SEQ ID NO: 1-30368. The polypeptides sequences are designated SEQ ID NO: 30369-60736. The nucleic acids and polypeptides are provided in the Sequence Listing. In the nucleic acids provided in the Sequence Listing, A is adenosine; C is cytosine; G is guanine; T is thymine; and N is any of the four bases. In the amino acids provided in the Sequence Listing, * corresponds to the stop codon.

The nucleic acid sequences of the present invention also include, nucleic acid sequences that hybridize to the complement of SEQ ID NO: 1-30368 under stringent hybridization conditions; nucleic acid sequences which are allelic variants or species homologues of any of the nucleic acid sequences recited above, or nucleic acid sequences that encode a peptide comprising a specific domain or truncation of the peptides encoded by SEQ ID NO: 1-30368. A polynucleotide comprising a nucleotide sequence having at least 90% identity to an identifying sequence of SEQ ID NO: 1-30368 or a degenerate variant or fragment thereof. The identifying sequence can be 100 base pairs in length.

The nucleic acid sequences of the present invention also include the sequence information from the nucleic acid sequences of SEQ ID NO: 1-30368. The sequence information can be a segment of any one of SEQ ID NO: 1-30368 that uniquely identifies or represents the sequence information of SEQ ID NO: 1-30368.

A collection as used in this application can be a collection of only one polynucleotide. The collection of sequence information or identifying information of each sequence can be provided on a nucleic acid array. In one embodiment, segments of sequence information is provided on a nucleic acid array to detect the polynucleotide that contains the segment. The array can be designed to detect full-match or mismatch to the polynucleotide that contains the segment. The collection can also be provided in a computer-readable format.

This invention also includes the reverse or direct complement of any of the nucleic acid sequences recited above; cloning or expression vectors containing the nucleic acid sequences; and host cells or organisms transformed with these expression vectors. Nucleic acid sequences (or their reverse or direct complements) according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology, such as use as hybridization probes, use as primers for PCR, use in an array, use in computer-readable media, use in sequencing

full-length genes, use for chromosome and gene mapping, use in the recombinant production of protein, and use in the generation of anti-sense DNA or RNA, their chemical analogs and the like.

In a preferred embodiment, the nucleic acid sequences of SEQ ID NO: 1-30368 or novel segments or parts of the nucleic acids of the invention are used as primers in expression assays that are well known in the art. In a particularly preferred embodiment, the nucleic acid sequences of SEQ ID NO: 1-30368 or novel segments or parts of the nucleic acids provided herein are used in diagnostics for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The isolated polynucleotides of the invention include, but are not limited to, a polynucleotide comprising any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368; a polynucleotide comprising any of the full length protein coding sequences of SEQ ID NO: 1-30368; and a polynucleotide comprising any of the nucleotide sequences of the mature protein coding sequences of SEQ ID NO: 1-30368. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent hybridization conditions to (a) the complement of any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368; (b) a nucleotide sequence encoding any one of the amino acid sequences set forth in the Sequence Listing (*e.g.*, SEQ ID NO: 30369-60736); (c) a polynucleotide which is an allelic variant of any polynucleotides recited above; (d) a polynucleotide which encodes a species homolog (*e.g.* orthologs) of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of any of the polypeptides comprising an amino acid sequence set forth in the Sequence Listing.

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising any of the amino acid sequences set forth in the Sequence Listing; or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides with biological activity that are encoded by (a) any of the polynucleotides having a nucleotide sequence set forth in SEQ ID NO: 1-30368; or (b) polynucleotides that hybridize to the complement of the polynucleotides of (a) under stringent hybridization conditions. Biologically or immunologically active variants of any of the polypeptide sequences in the Sequence Listing, and "substantial equivalents" thereof (*e.g.*, with at least about 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99% amino acid sequence identity) that preferably retain biological activity are also contemplated. The polypeptides of the invention may be wholly or partially chemically synthesized but are preferably produced by recombinant means using the genetically engineered cells (*e.g.* host cells) of the invention.

The invention also provides compositions comprising a polypeptide of the invention. Polypeptide compositions of the invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

5 The invention also provides host cells transformed or transfected with a polynucleotide of the invention.

The invention also relates to methods for producing a polypeptide of the invention comprising growing a culture of the host cells of the invention in a suitable culture medium under conditions permitting expression of the desired polypeptide, and purifying the polypeptide from the culture or from the host cells. Preferred embodiments include those in which the
10 protein produced by such process is a mature form of the protein.

Polynucleotides according to the invention have numerous applications in a variety of techniques known to those skilled in the art of molecular biology. These techniques include use as hybridization probes, use as oligomers, or primers, for PCR, use for chromosome and gene mapping, use in the recombinant production of protein, and use in generation of anti-sense DNA
15 or RNA, their chemical analogs and the like. For example, when the expression of an mRNA is largely restricted to a particular cell or tissue type, polynucleotides of the invention can be used as hybridization probes to detect the presence of the particular cell or tissue mRNA in a sample using, *e.g.*, *in situ* hybridization.

In other exemplary embodiments, the polynucleotides are used in diagnostics as
20 expressed sequence tags for identifying expressed genes or, as well known in the art and exemplified by Vollrath et al., Science 258:52-59 (1992), as expressed sequence tags for physical mapping of the human genome.

The polypeptides according to the invention can be used in a variety of conventional procedures and methods that are currently applied to other proteins. For example, a polypeptide
25 of the invention can be used to generate an antibody that specifically binds the polypeptide. Such antibodies, particularly monoclonal antibodies, are useful for detecting or quantitating the polypeptide in tissue. The polypeptides of the invention can also be used as molecular weight markers, and as a food supplement.

Methods are also provided for preventing, treating, or ameliorating a medical condition
30 which comprises the step of administering to a mammalian subject a therapeutically effective amount of a composition comprising a polypeptide of the present invention and a pharmaceutically acceptable carrier.

In particular, the polypeptides and polynucleotides of the invention can be utilized, for example, in methods for the prevention and/or treatment of disorders involving aberrant protein
35 expression or biological activity.

The present invention further relates to methods for detecting the presence of the polynucleotides or polypeptides of the invention in a sample. Such methods can, for example, be utilized as part of prognostic and diagnostic evaluation of disorders as recited herein and for the identification of subjects exhibiting a predisposition to such conditions. The invention provides

5 a method for detecting the polynucleotides of the invention in a sample, comprising contacting the sample with a compound that binds to and forms a complex with the polynucleotide of interest for a period sufficient to form the complex and under conditions sufficient to form a complex and detecting the complex such that if a complex is detected, the polynucleotide of interest is detected. The invention also provides a method for detecting the polypeptides of the
10 invention in a sample comprising contacting the sample with a compound that binds to and forms a complex with the polypeptide under conditions and for a period sufficient to form the complex and detecting the formation of the complex such that if a complex is formed, the polypeptide is detected.

The invention also provides kits comprising polynucleotide probes and/or monoclonal
15 antibodies, and optionally quantitative standards, for carrying out methods of the invention. Furthermore, the invention provides methods for evaluating the efficacy of drugs, and monitoring the progress of patients, involved in clinical trials for the treatment of disorders as recited above.

The invention also provides methods for the identification of compounds that modulate
20 (*i.e.*, increase or decrease) the expression or activity of the polynucleotides and/or polypeptides of the invention. Such methods can be utilized, for example, for the identification of compounds that can ameliorate symptoms of disorders as recited herein. Such methods can include, but are not limited to, assays for identifying compounds and other substances that interact with (*e.g.*, bind to) the polypeptides of the invention. The invention provides a method for identifying a
25 compound that binds to the polypeptides of the invention comprising contacting the compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a reporter gene sequence in the cell; and detecting the complex by detecting the reporter gene sequence expression such that if expression of the reporter gene is detected the compound that binds to a
30 polypeptide of the invention is identified.

The methods of the invention also provides methods for treatment which involve the administration of the polynucleotides or polypeptides of the invention to individuals exhibiting symptoms or tendencies. In addition, the invention encompasses methods for treating diseases or disorders as recited herein comprising administering compounds and other substances that
35 modulate the overall activity of the target gene products. Compounds and other substances can

effect such modulation either on the level of target gene/protein expression or target protein activity.

The polypeptides of the present invention and the polynucleotides encoding them are also useful for the same functions known to one of skill in the art as the polypeptides and polynucleotides to which they have homology (set forth in the sequence listing). If no homology is set forth for a sequence, then the polypeptides and polynucleotides of the present invention are useful for a variety of applications, as described herein, including use in arrays for detection.

4. DETAILED DESCRIPTION OF THE INVENTION

4.1 DEFINITIONS

It must be noted that as used herein and in the appended claims, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.

The term “active” refers to those forms of the polypeptide which retain the biologic and/or immunologic activities of any naturally occurring polypeptide. According to the invention, the terms “biologically active” or “biological activity” refer to a protein or peptide having structural, regulatory or biochemical functions of a naturally occurring molecule. Likewise “immunologically active” or “immunological activity” refers to the capability of the natural, recombinant or synthetic polypeptide to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The term “activated cells” as used in this application are those cells which are engaged in extracellular or intracellular membrane trafficking, including the export of secretory or enzymatic molecules as part of a normal or disease process.

The terms “complementary” or “complementarity” refer to the natural binding of polynucleotides by base pairing. For example, the sequence 5'-AGT-3' binds to the complementary sequence 3'-TCA-5'. Complementarity between two single-stranded molecules may be “partial” such that only some of the nucleic acids bind or it may be “complete” such that total complementarity exists between the single stranded molecules. The degree of complementarity between the nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands.

The term “embryonic stem cells (ES)” refers to a cell that can give rise to many differentiated cell types in an embryo or an adult, including the germ cells. The term “germ line stem cells (GSCs)” refers to stem cells derived from primordial stem cells that provide a steady and continuous source of germ cells for the production of gametes. The term “primordial germ

cells (PGCs)" refers to a small population of cells set aside from other cell lineages particularly from the yolk sac, mesenteries, or gonadal ridges during embryogenesis that have the potential to differentiate into germ cells and other cells. PGCs are the source from which GSCs and ES cells are derived. The PGCs, the GSCs and the ES cells are capable of self-renewal. Thus these cells not only populate the germ line and give rise to a plurality of terminally differentiated cells that comprise the adult specialized organs, but are able to regenerate themselves.

The term "expression modulating fragment," EMF, means a series of nucleotides which modulates the expression of an operably linked ORF or another EMF.

As used herein, a sequence is said to "modulate the expression of an operably linked sequence" when the expression of the sequence is altered by the presence of the EMF. EMFs include, but are not limited to, promoters, and promoter modulating sequences (inducible elements). One class of EMFs are nucleic acid fragments which induce the expression of an operably linked ORF in response to a specific regulatory factor or physiological event.

The terms "nucleotide sequence" or "nucleic acid" or "polynucleotide" or "oligonucleotide" are used interchangeably and refer to a heteropolymer of nucleotides or the sequence of these nucleotides. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA) or to any DNA-like or RNA-like material. In the sequences herein A is adenine, C is cytosine, T is thymine, G is guanine and N is A, C, G or T (U). It is contemplated that where the polynucleotide is RNA, the T (thymine) in the sequences provided herein is substituted with U (uracil). Generally, nucleic acid segments provided by this invention may be assembled from fragments of the genome and short oligonucleotide linkers, or from a series of oligonucleotides, or from individual nucleotides, to provide a synthetic nucleic acid which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon, or a eukaryotic gene.

The terms "oligonucleotide fragment" or a "polynucleotide fragment", "portion," or "segment" or "probe" or "primer" are used interchangeably and refer to a sequence of nucleotide residues which are at least about 5 nucleotides, more preferably at least about 7 nucleotides, more preferably at least about 9 nucleotides, more preferably at least about 11 nucleotides and most preferably at least about 17 nucleotides. The fragment is preferably less than about 500 nucleotides, preferably less than about 200 nucleotides, more preferably less than about 100 nucleotides, more preferably less than about 50 nucleotides and most preferably less than 30 nucleotides. Preferably the probe is from about 6 nucleotides to about 200 nucleotides, preferably from about 15 to about 50 nucleotides, more preferably from about 17 to 30 nucleotides and most preferably from about 20 to 25 nucleotides. Preferably the fragments can

be used in polymerase chain reaction (PCR), various hybridization procedures or microarray procedures to identify or amplify identical or related parts of mRNA or DNA molecules. A fragment or segment may uniquely identify each polynucleotide sequence of the present invention. Preferably the fragment comprises a sequence substantially similar to any one of SEQ ID NO: 1-30368.

Probes may, for example, be used to determine whether specific mRNA molecules are present in a cell or tissue or to isolate similar nucleic acid sequences from chromosomal DNA as described by Walsh et al. (Walsh, P.S. et al., 1992, PCR Methods Appl 1:241-250). They may be labeled by nick translation, Klenow fill-in reaction, PCR, or other methods well known in the art. Probes of the present invention, their preparation and/or labeling are elaborated in Sambrook, J. et al., 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY; or Ausubel, F.M. et al., 1989, Current Protocols in Molecular Biology, John Wiley & Sons, New York NY, both of which are incorporated herein by reference in their entirety.

The nucleic acid sequences of the present invention also include the sequence information from the nucleic acid sequences of SEQ ID NO: 1-30368. The sequence information can be a segment of any one of SEQ ID NO: 1-30368 that uniquely identifies or represents the sequence information of that sequence of SEQ ID NO: 1-30368. One such segment can be a twenty-mer nucleic acid sequence because the probability that a twenty-mer is fully matched in the human genome is 1 in 300. In the human genome, there are three billion base pairs in one set of chromosomes. Because 4^{20} possible twenty-mers exist, there are 300 times more twenty-mers than there are base pairs in a set of human chromosomes. Using the same analysis, the probability for a seventeen-mer to be fully matched in the human genome is approximately 1 in 5. When these segments are used in arrays for expression studies, fifteen-mer segments can be used. The probability that the fifteen-mer is fully matched in the expressed sequences is also approximately one in five because expressed sequences comprise less than approximately 5% of the entire genome sequence.

Similarly, when using sequence information for detecting a single mismatch, a segment can be a twenty-five mer. The probability that the twenty-five mer would appear in a human genome with a single mismatch is calculated by multiplying the probability for a full match ($1/4^{25}$) times the increased probability for mismatch at each nucleotide position (3×25). The probability that an eighteen mer with a single mismatch can be detected in an array for expression studies is approximately one in five. The probability that a twenty-mer with a single mismatch can be detected in a human genome is approximately one in five.

The term "open reading frame," ORF, means a series of nucleotide triplets coding for amino acids without any termination codons and is a sequence translatable into protein.

The terms "operably linked" or "operably associated" refer to functionally related nucleic acid sequences. For example, a promoter is operably associated or operably linked with a coding sequence if the promoter controls the transcription of the coding sequence. While operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements *e.g.* repressor genes are not contiguously linked to the coding sequence but still control transcription/translation of the coding sequence.

The term "pluripotent" refers to the capability of a cell to differentiate into a number of differentiated cell types that are present in an adult organism. A pluripotent cell is restricted in its differentiation capability in comparison to a totipotent cell.

The terms "polypeptide" or "peptide" or "amino acid sequence" refer to an oligopeptide, peptide, polypeptide or protein sequence or fragment thereof and to naturally occurring or synthetic molecules. A polypeptide "fragment," "portion," or "segment" is a stretch of amino acid residues of at least about 5 amino acids, preferably at least about 7 amino acids, more preferably at least about 9 amino acids and most preferably at least about 17 or more amino acids. The peptide preferably is not greater than about 200 amino acids, more preferably less than 150 amino acids and most preferably less than 100 amino acids. Preferably the peptide is from about 5 to about 200 amino acids. To be active, any polypeptide must have sufficient length to display biological and/or immunological activity.

The term "naturally occurring polypeptide" refers to polypeptides produced by cells that have not been genetically engineered and specifically contemplates various polypeptides arising from post-translational modifications of the polypeptide including, but not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation.

The term "translated protein coding portion" means a sequence which encodes for the full length protein which may include any leader sequence or any processing sequence.

The term "mature protein coding sequence" means a sequence which encodes a peptide or protein without a signal or leader sequence. The "mature protein portion" means that portion of the protein which does not include a signal or leader sequence. The peptide may have been produced by processing in the cell which removes any leader/signal sequence. The mature protein portion may or may not include an initial methionine residue. The methionine residue may be removed from the protein during processing in the cell. The peptide may be produced synthetically or the protein may have been produced using a polynucleotide only encoding for the mature protein coding sequence.

The term "derivative" refers to polypeptides chemically modified by such techniques as ubiquitination, labeling (*e.g.*, with radionuclides or various enzymes), covalent polymer attachment such as pegylation (derivatization with polyethylene glycol) and insertion or substitution by chemical synthesis of amino acids such as ornithine, which do not normally occur in human proteins.

The term "variant"(or "analog") refers to any polypeptide differing from naturally occurring polypeptides by amino acid insertions, deletions, and substitutions, created using, *e.g.*, recombinant DNA techniques. Guidance in determining which amino acid residues may be replaced, added or deleted without abolishing activities of interest, may be found by comparing the sequence of the particular polypeptide with that of homologous peptides and minimizing the number of amino acid sequence changes made in regions of high homology (conserved regions) or by replacing amino acids with consensus sequence.

Alternatively, recombinant variants encoding these same or similar polypeptides may be synthesized or selected by making use of the "redundancy" in the genetic code. Various codon substitutions, such as the silent changes which produce various restriction sites, may be introduced to optimize cloning into a plasmid or viral vector or expression in a particular prokaryotic or eukaryotic system. Mutations in the polynucleotide sequence may be reflected in the polypeptide or domains of other peptides added to the polypeptide to modify the properties of any part of the polypeptide, to change characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate.

Preferably, amino acid "substitutions" are the result of replacing one amino acid with another amino acid having similar structural and/or chemical properties, *i.e.*, conservative amino acid replacements. "Conservative" amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved. For example, nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; positively charged (basic) amino acids include arginine, lysine, and histidine; and negatively charged (acidic) amino acids include aspartic acid and glutamic acid. "Insertions" or "deletions" are preferably in the range of about 1 to 20 amino acids, more preferably 1 to 10 amino acids. The variation allowed may be experimentally determined by systematically making insertions, deletions, or substitutions of amino acids in a polypeptide molecule using recombinant DNA techniques and assaying the resulting recombinant variants for activity.

Alternatively, where alteration of function is desired, insertions, deletions or non-conservative alterations can be engineered to produce altered polypeptides. Such alterations

can, for example, alter one or more of the biological functions or biochemical characteristics of the polypeptides of the invention. For example, such alterations may change polypeptide characteristics such as ligand-binding affinities, interchain affinities, or degradation/turnover rate. Further, such alterations can be selected so as to generate polypeptides that are better suited for expression, scale up and the like in the host cells chosen for expression. For example, cysteine residues can be deleted or substituted with another amino acid residue in order to eliminate disulfide bridges.

The terms "purified" or "substantially purified" as used herein denotes that the indicated nucleic acid or polypeptide is present in the substantial absence of other biological macromolecules, *e.g.*, polynucleotides, proteins, and the like. In one embodiment, the polynucleotide or polypeptide is purified such that it constitutes at least 95% by weight, more preferably at least 99% by weight, of the indicated biological macromolecules present (but water, buffers, and other small molecules, especially molecules having a molecular weight of less than 1000 daltons, can be present).

The term "isolated" as used herein refers to a nucleic acid or polypeptide separated from at least one other component (*e.g.*, nucleic acid or polypeptide) present with the nucleic acid or polypeptide in its natural source. In one embodiment, the nucleic acid or polypeptide is found in the presence of (if anything) only a solvent, buffer, ion, or other component normally present in a solution of the same. The terms "isolated" and "purified" do not encompass nucleic acids or polypeptides present in their natural source.

The term "recombinant," when used herein to refer to a polypeptide or protein, means that a polypeptide or protein is derived from recombinant (*e.g.*, microbial, insect, or mammalian) expression systems. "Microbial" refers to recombinant polypeptides or proteins made in bacterial or fungal (*e.g.*, yeast) expression systems. As a product, "recombinant microbial" defines a polypeptide or protein essentially free of native endogenous substances and unaccompanied by associated native glycosylation. Polypeptides or proteins expressed in most bacterial cultures, *e.g.*, *E. coli*, will be free of glycosylation modifications; polypeptides or proteins expressed in yeast will have a glycosylation pattern in general different from those expressed in mammalian cells.

The term "recombinant expression vehicle or vector" refers to a plasmid or phage or virus or vector, for expressing a polypeptide from a DNA (RNA) sequence. An expression vehicle can comprise a transcriptional unit comprising an assembly of (1) a genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers, (2) a structural or coding sequence which is transcribed into mRNA and translated into protein, and (3) appropriate transcription initiation and termination sequences. Structural units intended for use

in yeast or eukaryotic expression systems preferably include a leader sequence enabling extracellular secretion of translated protein by a host cell. Alternatively, where recombinant protein is expressed without a leader or transport sequence, it may include an amino terminal methionine residue. This residue may or may not be subsequently cleaved from the expressed recombinant protein to provide a final product.

The term "recombinant expression system" means host cells which have stably integrated a recombinant transcriptional unit into chromosomal DNA or carry the recombinant transcriptional unit extrachromosomally. Recombinant expression systems as defined herein will express heterologous polypeptides or proteins upon induction of the regulatory elements linked to the DNA segment or synthetic gene to be expressed. This term also means host cells which have stably integrated a recombinant genetic element or elements having a regulatory role in gene expression, for example, promoters or enhancers. Recombinant expression systems as defined herein will express polypeptides or proteins endogenous to the cell upon induction of the regulatory elements linked to the endogenous DNA segment or gene to be expressed. The cells can be prokaryotic or eukaryotic.

The term "secreted" includes a protein that is transported across or through a membrane, including transport as a result of signal sequences in its amino acid sequence when it is expressed in a suitable host cell. "Secreted" proteins include without limitation proteins secreted wholly (*e.g.*, soluble proteins) or partially (*e.g.*, receptors) from the cell in which they are expressed. "Secreted" proteins also include without limitation proteins that are transported across the membrane of the endoplasmic reticulum. "Secreted" proteins are also intended to include proteins containing non-typical signal sequences (*e.g.* Interleukin-1 Beta, see Krasney, P.A. and Young, P.R. (1992) Cytokine 4(2):134 -143) and factors released from damaged cells (*e.g.* Interleukin-1 Receptor Antagonist, see Arend, W.P. et. al. (1998) Annu. Rev. Immunol. 16:27-55)

Where desired, an expression vector may be designed to contain a "signal or leader sequence" which will direct the polypeptide through the membrane of a cell. Such a sequence may be naturally present on the polypeptides of the present invention or provided from heterologous protein sources by recombinant DNA techniques.

The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Stringent conditions can include highly stringent conditions (*i.e.*, hybridization to filter-bound DNA in 0.5 M NaHPO₄, 7% sodium dodecyl sulfate (SDS), 1 mM EDTA at 65°C, and washing in 0.1X SSC/0.1% SDS at 68°C), and moderately stringent conditions (*i.e.*, washing in 0.2X SSC/0.1% SDS at 42°C). Other exemplary hybridization conditions are described herein in the examples.

In instances of hybridization of deoxyoligonucleotides, additional exemplary stringent hybridization conditions include washing in 6X SSC/0.05% sodium pyrophosphate at 37°C (for 14-base oligonucleotides), 48°C (for 17-base oligos), 55°C (for 20-base oligonucleotides), and 60°C (for 23-base oligonucleotides).

5 As used herein, "substantially equivalent" can refer both to nucleotide and amino acid sequences, for example a mutant sequence, that varies from a reference sequence by one or more substitutions, deletions, or additions, the net effect of which does not result in an adverse functional dissimilarity between the reference and subject sequences. Typically, such a substantially equivalent sequence varies from one of those listed herein by no more than about
10 35% (*i.e.*, the number of individual residue substitutions, additions, and/or deletions in a substantially equivalent sequence, as compared to the corresponding reference sequence, divided by the total number of residues in the substantially equivalent sequence is about 0.35 or less). Such a sequence is said to have 65% sequence identity to the listed sequence. In one embodiment, a substantially equivalent, *e.g.*, mutant, sequence of the invention varies from a
15 listed sequence by no more than 30% (70% sequence identity); in a variation of this embodiment, by no more than 25% (75% sequence identity); and in a further variation of this embodiment, by no more than 20% (80% sequence identity) and in a further variation of this embodiment, by no more than 10% (90% sequence identity) and in a further variation of this embodiment, by no more than 5% (95% sequence identity). Substantially equivalent, *e.g.*,
20 mutant, amino acid sequences according to the invention preferably have at least 80% sequence identity with a listed amino acid sequence, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% identity, more preferably at least 98% identity, and most preferably at least 99% identity. Substantially equivalent nucleotide sequences of the invention can have lower percent sequence identities, taking into
25 account, for example, the redundancy or degeneracy of the genetic code. Preferably, nucleotide sequence has at least about 65% identity, more preferably at least about 75% identity, more preferably at least about 80% sequence identity, more preferably at least about 85% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% identity, more preferably at least about 98% sequence identity, and most preferably at least
30 about 99% sequence identity. For the purposes of the present invention, sequences having substantially equivalent biological activity and substantially equivalent expression characteristics are considered substantially equivalent. For the purposes of determining equivalence, truncation of the mature sequence (*e.g.*, via a mutation which creates a spurious stop codon) should be disregarded. Sequence identity may be determined, *e.g.*, using the Jotun Hein method (Hein, J.

(1990) Methods Enzymol. 183:626-645). Identity between sequences can also be determined by other methods known in the art, *e.g.* by varying hybridization conditions.

The term "totipotent" refers to the capability of a cell to differentiate into all of the cell types of an adult organism.

5 The term "transformation" means introducing DNA into a suitable host cell so that the DNA is replicable, either as an extrachromosomal element, or by chromosomal integration. The term "transfection" refers to the taking up of an expression vector by a suitable host cell, whether or not any coding sequences are in fact expressed. The term "infection" refers to the introduction of nucleic acids into a suitable host cell by use of a virus or viral vector.

10 As used herein, an "uptake modulating fragment," UMF, means a series of nucleotides which mediate the uptake of a linked DNA fragment into a cell. UMFs can be readily identified using known UMFs as a target sequence or target motif with the computer-based systems described below. The presence and activity of a UMF can be confirmed by attaching the suspected UMF to a marker sequence. The resulting nucleic acid molecule is then incubated
15 with an appropriate host under appropriate conditions and the uptake of the marker sequence is determined. As described above, a UMF will increase the frequency of uptake of a linked marker sequence.

Each of the above terms is meant to encompass all that is described for each, unless the context dictates otherwise.

20 4.2 NUCLEIC ACIDS OF THE INVENTION

Nucleotide sequences of the invention are set forth in the Sequence Listing.

The isolated polynucleotides of the invention include a polynucleotide comprising the nucleotide sequences of SEQ ID NO: 1-30368; a polynucleotide encoding any one of the peptide
25 sequences of SEQ ID NO: 30369-60736; and a polynucleotide comprising the nucleotide sequence encoding the mature protein coding sequence of the polypeptides of any one of SEQ ID NO: 30369-60736. The polynucleotides of the present invention also include, but are not limited to, a polynucleotide that hybridizes under stringent conditions to (a) the complement of any of the nucleotides sequences of SEQ ID NO: 1-30368; (b) nucleotide sequences encoding any one
30 of the amino acid sequences set forth in the Sequence Listing; (c) a polynucleotide which is an allelic variant of any polynucleotide recited above; (d) a polynucleotide which encodes a species homolog of any of the proteins recited above; or (e) a polynucleotide that encodes a polypeptide comprising a specific domain or truncation of the polypeptides of SEQ ID NO: 30369-60736. Domains of interest may depend on the nature of the encoded polypeptide; *e.g.*, domains in
35 receptor-like polypeptides include ligand-binding, extracellular, transmembrane, or cytoplasmic

domains, or combinations thereof; domains in immunoglobulin-like proteins include the variable immunoglobulin-like domains; domains in enzyme-like polypeptides include catalytic and substrate binding domains; and domains in ligand polypeptides include receptor-binding domains.

5 The polynucleotides of the invention include naturally occurring or wholly or partially synthetic DNA, *e.g.*, cDNA and genomic DNA, and RNA, *e.g.*, mRNA. The polynucleotides may include all of the coding region of the cDNA or may represent a portion of the coding region of the cDNA.

10 The present invention also provides genes corresponding to the cDNA sequences disclosed herein. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include the preparation of probes or primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. Further 5' and 3' sequence can be obtained using methods known in the art. For example, full length cDNA or genomic DNA that
15 corresponds to any of the polynucleotides of SEQ ID NO: 1-30368 can be obtained by screening appropriate cDNA or genomic DNA libraries under suitable hybridization conditions using any of the polynucleotides of SEQ ID NO: 1-30368 or a portion thereof as a probe. Alternatively, the polynucleotides of SEQ ID NO: 1-30368 may be used as the basis for suitable primer(s) that allow identification and/or amplification of genes in appropriate genomic DNA or cDNA libraries.

20 The nucleic acid sequences of the invention can be assembled from ESTs and sequences (including cDNA and genomic sequences) obtained from one or more public databases, such as dbEST, gbpr, and UniGene. The EST sequences can provide identifying sequence information, representative fragment or segment information, or novel segment information for the full-length gene.

25 The polynucleotides of the invention also provide polynucleotides including nucleotide sequences that are substantially equivalent to the polynucleotides recited above. Polynucleotides according to the invention can have, *e.g.*, at least about 65%, at least about 70%, at least about 75%, at least about 80%, 81%, 82%, 83%, 84%, more typically at least about 85%, 86%, 87%, 88%, 89%, more typically at least about 90%, 91%, 92%, 93%, 94%, and even more typically at
30 least about 95%, 96%, 97%, 98%, 99%, sequence identity to a polynucleotide recited above.

 Included within the scope of the nucleic acid sequences of the invention are nucleic acid sequence fragments that hybridize under stringent conditions to any of the nucleotide sequences of SEQ ID NO: 1-30368, or complements thereof, which fragment is greater than about 5 nucleotides, preferably 7 nucleotides, more preferably greater than 9 nucleotides and most
35 preferably greater than 17 nucleotides. Fragments of, *e.g.* 15, 17, or 20 nucleotides or more that

are selective for (*i.e.* specifically hybridize to any one of the polynucleotides of the invention) are contemplated. Probes capable of specifically hybridizing to a polynucleotide can differentiate polynucleotide sequences of the invention from other polynucleotide sequences in the same family of genes or can differentiate human genes from genes of other species, and are preferably based on unique nucleotide sequences.

The sequences falling within the scope of the present invention are not limited to these specific sequences, but also include allelic and species variations thereof. Allelic and species variations can be routinely determined by comparing the sequence provided in SEQ ID NO: 1-30368, a representative fragment thereof, or a nucleotide sequence at least 90% identical, preferably 95% identical, to SEQ ID NO: 1-30368 with a sequence from another isolate of the same species. Furthermore, to accommodate codon variability, the invention includes nucleic acid molecules coding for the same amino acid sequences as do the specific ORFs disclosed herein. In other words, in the coding region of an ORF, substitution of one codon for another codon that encodes the same amino acid is expressly contemplated.

The nearest neighbor or homology result for the nucleic acids of the present invention, including SEQ ID NO: 1-30368 can be obtained by searching a database using an algorithm or a program. Preferably, a BLAST which stands for Basic Local Alignment Search Tool is used to search for local sequence alignments (Altschul, S.F. J Mol. Evol. 36 290-300 (1993) and Altschul S.F. et al. J. Mol. Biol. 21:403-410 (1990)). Alternatively a FASTA version 3 search against Genpept, using Fastxy algorithm.

Species homologs (or orthologs) of the disclosed polynucleotides and proteins are also provided by the present invention. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous or related to that encoded by the polynucleotides.

The nucleic acid sequences of the invention are further directed to sequences which encode variants of the described nucleic acids. These amino acid sequence variants may be prepared by methods known in the art by introducing appropriate nucleotide changes into a native or variant polynucleotide. There are two variables in the construction of amino acid sequence variants: the location of the mutation and the nature of the mutation. Nucleic acids encoding the amino acid sequence variants are preferably constructed by mutating the polynucleotide to encode an amino acid sequence that does not occur in nature. These nucleic

acid alterations can be made at sites that differ in the nucleic acids from different species (variable positions) or in highly conserved regions (constant regions). Sites at such locations will typically be modified in series, *e.g.*, by substituting first with conservative choices (*e.g.*, hydrophobic amino acid to a different hydrophobic amino acid) and then with more distant choices (*e.g.*, hydrophobic amino acid to a charged amino acid), and then deletions or insertions may be made at the target site. Amino acid sequence deletions generally range from about 1 to 30 residues, preferably about 1 to 10 residues, and are typically contiguous. Amino acid insertions include amino- and/or carboxyl-terminal fusions ranging in length from one to one hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Intrasequence insertions may range generally from about 1 to 10 amino residues, preferably from 1 to 5 residues. Examples of terminal insertions include the heterologous signal sequences necessary for secretion or for intracellular targeting in different host cells and sequences such as FLAG or poly-histidine sequences useful for purifying the expressed protein.

In a preferred method, polynucleotides encoding the novel amino acid sequences are changed via site-directed mutagenesis. This method uses oligonucleotide sequences to alter a polynucleotide to encode the desired amino acid variant, as well as sufficient adjacent nucleotides on both sides of the changed amino acid to form a stable duplex on either side of the site of being changed. In general, the techniques of site-directed mutagenesis are well known to those of skill in the art and this technique is exemplified by publications such as, Edelman et al., *DNA* 2:183 (1983). A versatile and efficient method for producing site-specific changes in a polynucleotide sequence was published by Zoller and Smith, *Nucleic Acids Res.* 10:6487-6500 (1982). PCR may also be used to create amino acid sequence variants of the novel nucleic acids. When small amounts of template DNA are used as starting material, primer(s) that differs slightly in sequence from the corresponding region in the template DNA can generate the desired amino acid variant. PCR amplification results in a population of product DNA fragments that differ from the polynucleotide template encoding the polypeptide at the position specified by the primer. The product DNA fragments replace the corresponding region in the plasmid and this gives a polynucleotide encoding the desired amino acid variant.

A further technique for generating amino acid variants is the cassette mutagenesis technique described in Wells et al., *Gene* 34:315 (1985); and other mutagenesis techniques well known in the art, such as, for example, the techniques in Sambrook et al., *supra*, and *Current Protocols in Molecular Biology*, Ausubel et al. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be used in the practice of the invention for the cloning and expression

of these novel nucleic acids. Such DNA sequences include those which are capable of hybridizing to the appropriate novel nucleic acid sequence under stringent conditions.

Polynucleotides encoding preferred polypeptide truncations of the invention can be used to generate polynucleotides encoding chimeric or fusion proteins comprising one or more domains of the invention and heterologous protein sequences.

The polynucleotides of the invention additionally include the complement of any of the polynucleotides recited above. The polynucleotide can be DNA (genomic, cDNA, amplified, or synthetic) or RNA. Methods and algorithms for obtaining such polynucleotides are well known to those of skill in the art and can include, for example, methods for determining hybridization conditions that can routinely isolate polynucleotides of the desired sequence identities.

In accordance with the invention, polynucleotide sequences comprising the mature protein coding sequences corresponding to any one of SEQ ID NO: 1-30368, or functional equivalents thereof, may be used to generate recombinant DNA molecules that direct the expression of that nucleic acid, or a functional equivalent thereof, in appropriate host cells. Also included are the cDNA inserts of any of the clones identified herein.

A polynucleotide according to the invention can be joined to any of a variety of other nucleotide sequences by well-established recombinant DNA techniques (see Sambrook J et al. (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, NY). Useful nucleotide sequences for joining to polynucleotides include an assortment of vectors, *e.g.*, plasmids, cosmids, lambda phage derivatives, phagemids, and the like, that are well known in the art. Accordingly, the invention also provides a vector including a polynucleotide of the invention and a host cell containing the polynucleotide. In general, the vector contains an origin of replication functional in at least one organism, convenient restriction endonuclease sites, and a selectable marker for the host cell. Vectors according to the invention include expression vectors, replication vectors, probe generation vectors, and sequencing vectors. A host cell according to the invention can be a prokaryotic or eukaryotic cell and can be a unicellular organism or part of a multicellular organism.

The present invention further provides recombinant constructs comprising a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1-30368 or a fragment thereof or any other polynucleotides of the invention. In one embodiment, the recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a nucleic acid having any of the nucleotide sequences of SEQ ID NO: 1-30368 or a fragment thereof is inserted, in a forward or reverse orientation. In the case of a vector comprising one of the ORFs of the present invention, the vector may further comprise regulatory sequences, including for example, a promoter, operably linked to the ORF. Large numbers of suitable vectors and

promoters are known to those of skill in the art and are commercially available for generating the recombinant constructs of the present invention. The following vectors are provided by way of example. Bacterial: pBs, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia).

5 Eukaryotic: pWLneo, pSV2cat, pOG44, PXTI, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia).

The isolated polynucleotide of the invention may be operably linked to an expression control sequence such as the pMT2 or pED expression vectors disclosed in Kaufman et al., *Nucleic Acids Res.* 19, 4485-4490 (1991), in order to produce the protein recombinantly. Many
10 suitable expression control sequences are known in the art. General methods of expressing recombinant proteins are also known and are exemplified in R. Kaufman, *Methods in Enzymology* 185, 537-566 (1990). As defined herein "operably linked" means that the isolated polynucleotide of the invention and an expression control sequence are situated within a vector or cell in such a way that the protein is expressed by a host cell which has been transformed
15 (transfected) with the ligated polynucleotide/expression control sequence.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda PR, and trc. Eukaryotic promoters include CMV immediate early, HSV thymidine
20 kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art. Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of *E. coli* and *S. cerevisiae* TRP1 gene, and a promoter derived from a highly-expressed gene to direct
25 transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), a-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the
30 periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an amino terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product. Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination
35 signals in operable reading phase with a functional promoter. The vector will comprise one or

more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli*, *Bacillus subtilis*, *Salmonella typhimurium* and various species within the genera *Pseudomonas*, *Streptomyces*, and *Staphylococcus*, although others may also be employed as a matter of choice.

As a representative but non-limiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM 1 (Promega Biotech, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced or derepressed by appropriate means (*e.g.*, temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Polynucleotides of the invention can also be used to induce immune responses. For example, as described in Fan et al., *Nat. Biotech.* 17:870-872 (1999), incorporated herein by reference, nucleic acid sequences encoding a polypeptide may be used to generate antibodies against the encoded polypeptide following topical administration of naked plasmid DNA or following injection, and preferably intramuscular injection of the DNA. The nucleic acid sequences are preferably inserted in a recombinant expression vector and may be in the form of naked DNA.

4.3 ANTISENSE

Another aspect of the invention pertains to isolated antisense nucleic acid molecules that are hybridizable to or complementary to the nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1-30368, or fragments, analogs or derivatives thereof. An "antisense" nucleic acid comprises a nucleotide sequence that is complementary to a "sense" nucleic acid encoding a protein, *e.g.*, complementary to the coding strand of a double-stranded cDNA molecule or complementary to an mRNA sequence. In specific aspects, antisense nucleic acid molecules are provided that comprise a sequence complementary to at least about 10, 25, 50, 100, 250 or 500 nucleotides or an entire coding strand, or to only a portion thereof. Nucleic acid molecules encoding fragments, homologs, derivatives and analogs of a protein of any of SEQ ID

NO: 30369-60736 or antisense nucleic acids complementary to a nucleic acid sequence of SEQ ID NO: 1-30368 are additionally provided.

In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence of the invention. The term "coding region" refers to the region of the nucleotide sequence comprising codons which are translated into amino acid residues. In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence of the invention. The term "noncoding region" refers to 5' and 3' sequences which flank the coding region that are not translated into amino acids (*i.e.*, also referred to as 5' and 3' untranslated regions).

Given the coding strand sequences encoding a nucleic acid disclosed herein (*e.g.*, SEQ ID NO: 1-30368), antisense nucleic acids of the invention can be designed according to the rules of Watson and Crick or Hoogsteen base pairing. The antisense nucleic acid molecule can be complementary to the entire coding region of a mRNA, but more preferably is an oligonucleotide that is antisense to only a portion of the coding or noncoding region of a mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of a mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. An antisense nucleic acid of the invention can be constructed using chemical synthesis or enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (*e.g.*, an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, *e.g.*, phosphorothioate derivatives and acridine substituted nucleotides can be used.

Examples of modified nucleotides that can be used to generate the antisense nucleic acid include: 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxymethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (ν), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (ν), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3) ν , and 2,6-diaminopurine. Alternatively, the

antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (*i.e.*, RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

5 The antisense nucleic acid molecules of the invention are typically administered to a subject or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a protein according to the invention to thereby inhibit expression of the protein, *e.g.*, by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of
10 an antisense nucleic acid molecule that binds to DNA duplexes, through specific interactions in the major groove of the double helix. An example of a route of administration of antisense nucleic acid molecules of the invention includes direct injection at a tissue site. Alternatively, antisense nucleic acid molecules can be modified to target selected cells and then administered systemically. For example, for systemic administration, antisense molecules can be modified
15 such that they specifically bind to receptors or antigens expressed on a selected cell surface, *e.g.*, by linking the antisense nucleic acid molecules to peptides or antibodies that bind to cell surface receptors or antigens. The antisense nucleic acid molecules can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the
20 control of a strong pol II or pol III promoter are preferred.

In yet another embodiment, the antisense nucleic acid molecule of the invention is an α -anomeric nucleic acid molecule. An α -anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β -units, the strands run parallel to each other (Gaultier *et al.* (1987) *Nucleic Acids Res* 15: 6625-6641). The
25 antisense nucleic acid molecule can also comprise a 2'-O-methylribonucleotide (Inoue *et al.* (1987) *Nucleic Acids Res* 15: 6131-6148) or a chimeric RNA-DNA analogue (Inoue *et al.* (1987) *FEBS Lett* 215: 327-330).

4.4 RIBOZYMES AND PNA MOIETIES

30 In still another embodiment, an antisense nucleic acid of the invention is a ribozyme. Ribozymes are catalytic RNA molecules with ribonuclease activity that are capable of cleaving a single-stranded nucleic acid, such as a mRNA, to which they have a complementary region. Thus, ribozymes (*e.g.*, hammerhead ribozymes (described in Haselhoff and Gerlach (1988) *Nature* 334:585-591)) can be used to catalytically cleave a mRNA transcripts to thereby inhibit
35 translation of a mRNA. A ribozyme having specificity for a nucleic acid of the invention can be

designed based upon the nucleotide sequence of a DNA disclosed herein (*i.e.*, SEQ ID NO: 1-30368). For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in an mRNA of SEQ ID NO: 1-30368 (see, *e.g.*, Cech *et al.* U.S. Pat. No. 4,987,071; and Cech *et al.* U.S. Pat. No. 5,116,742). Alternatively, polynucleotides of the invention can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, *e.g.*, Bartel *et al.*, (1993) *Science* 261:1411-1418.

Alternatively, gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region (*e.g.*, promoter and/or enhancers) to form triple helical structures that prevent transcription of the gene in target cells. See generally, Helene. (1991) *Anticancer Drug Des.* 6: 569-84; Helene. *et al.* (1992) *Ann. N.Y. Acad. Sci.* 660:27-36; and Maher (1992) *Bioassays* 14: 807-15.

In various embodiments, the nucleic acids of the invention can be modified at the base moiety, sugar moiety or phosphate backbone to improve, *e.g.*, the stability, hybridization, or solubility of the molecule. For example, the deoxyribose phosphate backbone of the nucleic acids can be modified to generate peptide nucleic acids (see Hyrup *et al.* (1996) *Bioorg Med Chem* 4: 5-23). As used herein, the terms "peptide nucleic acids" or "PNAs" refer to nucleic acid mimics, *e.g.*, DNA mimics, in which the deoxyribose phosphate backbone is replaced by a pseudopeptide backbone and only the four natural nucleobases are retained. The neutral backbone of PNAs has been shown to allow for specific hybridization to DNA and RNA under conditions of low ionic strength. The synthesis of PNA oligomers can be performed using standard solid phase peptide synthesis protocols as described in Hyrup *et al.* (1996) above; Perry-O'Keefe *et al.* (1996) *PNAS* 93: 14670-675.

PNAs of the invention can be used in therapeutic and diagnostic applications. For example, PNAs can be used as antisense or antigene agents for sequence-specific modulation of gene expression by, *e.g.*, inducing transcription or translation arrest or inhibiting replication. PNAs of the invention can also be used, *e.g.*, in the analysis of single base pair mutations in a gene by, *e.g.*, PNA directed PCR clamping; as artificial restriction enzymes when used in combination with other enzymes, *e.g.*, S1 nucleases (Hyrup B. (1996) above); or as probes or primers for DNA sequence and hybridization (Hyrup *et al.* (1996), above; Perry-O'Keefe (1996), above).

In another embodiment, PNAs of the invention can be modified, *e.g.*, to enhance their stability or cellular uptake, by attaching lipophilic or other helper groups to PNA, by the formation of PNA-DNA chimeras, or by the use of liposomes or other techniques of drug delivery known in the art. For example, PNA-DNA chimeras can be generated that may

combine the advantageous properties of PNA and DNA. Such chimeras allow DNA recognition enzymes, *e.g.*, RNase H and DNA polymerases, to interact with the DNA portion while the PNA portion would provide high binding affinity and specificity. PNA-DNA chimeras can be linked using linkers of appropriate lengths selected in terms of base stacking, number of bonds between the nucleobases, and orientation (Hyrup (1996) above). The synthesis of PNA-DNA chimeras can be performed as described in Hyrup (1996) above and Finn *et al.* (1996) *Nucl Acids Res* 24: 3357-63. For example, a DNA chain can be synthesized on a solid support using standard phosphoramidite coupling chemistry, and modified nucleoside analogs, *e.g.*, 5'-(4-methoxytrityl)amino-5'-deoxy-thymidine phosphoramidite, can be used between the PNA and the 5' end of DNA (Mag *et al.* (1989) *Nucl Acid Res* 17: 5973-88). PNA monomers are then coupled in a stepwise manner to produce a chimeric molecule with a 5' PNA segment and a 3' DNA segment (Finn *et al.* (1996) above). Alternatively, chimeric molecules can be synthesized with a 5' DNA segment and a 3' PNA segment. See, Petersen *et al.* (1975) *Bioorg Med Chem Lett* 5: 1119-11124.

In other embodiments, the oligonucleotide may include other appended groups such as peptides (*e.g.*, for targeting host cell receptors *in vivo*), or agents facilitating transport across the cell membrane (see, *e.g.*, Letsinger *et al.*, 1989, *Proc. Natl. Acad. Sci. U.S.A.* 86:6553-6556; Lemaitre *et al.*, 1987, *Proc. Natl. Acad. Sci.* 84:648-652; PCT Publication No. W088/09810) or the blood-brain barrier (see, *e.g.*, PCT Publication No. W089/10134). In addition, oligonucleotides can be modified with hybridization triggered cleavage agents (See, *e.g.*, Krol *et al.*, 1988, *BioTechniques* 6:958-976) or intercalating agents (see, *e.g.*, Zon, 1988, *Pharm. Res.* 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, *e.g.*, a peptide, a hybridization triggered cross-linking agent, a transport agent, a hybridization-triggered cleavage agent, etc.

4.5 HOSTS

The present invention further provides host cells genetically engineered to contain the polynucleotides of the invention. For example, such host cells may contain nucleic acids of the invention introduced into the host cell using known transformation, transfection or infection methods. The present invention still further provides host cells genetically engineered to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in the cell.

Knowledge of nucleic acid sequences allows for modification of cells to permit, or increase, expression of endogenous polypeptide. Cells can be modified (*e.g.*, by homologous

recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the polypeptide at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the encoding sequences. See, for example, PCT International Publication No. WO94/12650, PCT International Publication No. WO92/20808, and PCT International Publication No. WO91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.*, *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

The host cell can be a higher eukaryotic host cell, such as a mammalian cell, a lower eukaryotic host cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the recombinant construct into the host cell can be effected by calcium phosphate transfection, DEAE, dextran mediated transfection, or electroporation (Davis, L. et al., *Basic Methods in Molecular Biology* (1986)). The host cells containing one of the polynucleotides of the invention, can be used in conventional manners to produce the gene product encoded by the isolated fragment (in the case of an ORF) or can be used to produce a heterologous protein under the control of the EMF.

Any host/vector system can be used to express one or more of the ORFs of the present invention. These include, but are not limited to, eukaryotic hosts such as HeLa cells, Cv-1 cell, COS cells, 293 cells, and Sf9 cells, as well as prokaryotic host such as *E. coli* and *B. subtilis*. The most preferred cells are those which do not normally express the particular polypeptide or protein or which expresses the polypeptide or protein at low natural level. Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*, Second Edition, Cold Spring Harbor, New York (1989), the disclosure of which is hereby incorporated by reference.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell* 23:175 (1981). Other cell lines capable of expressing a compatible vector are, for example, the C127, monkey COS cells, Chinese Hamster Ovary (CHO) cells, human kidney 293 cells, human epidermal A431 cells, human Colo205 cells, 3T3

cells, CV-1 cells, other transformed primate cell lines, normal diploid cells, cell strains derived from *in vitro* culture of primary tissue, primary explants, HeLa cells, mouse L cells, BHK, HL-60, U937, HaK or Jurkat cells. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements. Recombinant polypeptides and proteins produced in bacterial culture are usually isolated by initial extraction from cell pellets, followed by one or more salting-out, aqueous ion exchange or size exclusion chromatography steps. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps. Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents.

Alternatively, it may be possible to produce the protein in lower eukaryotes such as yeast or insects or in prokaryotes such as bacteria. Potentially suitable yeast strains include *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Kluyveromyces* strains, *Candida*, or any yeast strain capable of expressing heterologous proteins. Potentially suitable bacterial strains include *Escherichia coli*, *Bacillus subtilis*, *Salmonella typhimurium*, or any bacterial strain capable of expressing heterologous proteins. If the protein is made in yeast or bacteria, it may be necessary to modify the protein produced therein, for example by phosphorylation or glycosylation of the appropriate sites, in order to obtain the functional protein. Such covalent attachments may be accomplished using known chemical or enzymatic methods.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequence include polyadenylation signals, mRNA stability elements, splice

sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the host cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (*gpt*) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultchi et al., each of which is incorporated by reference herein in its entirety.

4.6 POLYPEPTIDES OF THE INVENTION

The isolated polypeptides of the invention include, but are not limited to, a polypeptide comprising: the amino acid sequences set forth as any one of SEQ ID NO: 30369-60736 or an amino acid sequence encoded by any one of the nucleotide sequences SEQ ID NO: 1-30368 or the corresponding full length or mature protein. Polypeptides of the invention also include polypeptides preferably with biological or immunological activity that are encoded by: (a) a polynucleotide having any one of the nucleotide sequences set forth in SEQ ID NO: 1-30368 or

(b) polynucleotides encoding any one of the amino acid sequences set forth as SEQ ID NO: 30369-60736 or (c) polynucleotides that hybridize to the complement of the polynucleotides of either (a) or (b) under stringent hybridization conditions. The invention also provides biologically active or immunologically active variants of any of the amino acid sequences set forth as SEQ ID NO: 30369-60736 or the corresponding full length or mature protein; and "substantial equivalents" thereof (*e.g.*, with at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, 86%, 87%, 88%, 89%, at least about 90%, 91%, 92%, 93%, 94%, typically at least about 95%, 96%, 97%, more typically at least about 98%, or most typically at least about 99% amino acid identity) that retain biological activity.

Polypeptides encoded by allelic variants may have a similar, increased, or decreased activity compared to polypeptides comprising SEQ ID NO: 30369-60736.

Fragments of the proteins of the present invention which are capable of exhibiting biological activity are also encompassed by the present invention. Fragments of the protein may be in linear form or they may be cyclized using known methods, for example, as described in H. U. Saragovi, et al., *Bio/Technology* 10, 773-778 (1992) and in R. S. McDowell, et al., *J. Amer. Chem. Soc.* 114, 9245-9253 (1992), both of which are incorporated herein by reference. Such fragments may be fused to carrier molecules such as immunoglobulins for many purposes, including increasing the valency of protein binding sites.

The present invention also provides both full-length and mature forms (for example, without a signal sequence or precursor sequence) of the disclosed proteins. The protein coding sequence is identified in the sequence listing by translation of the disclosed nucleotide sequences. The mature form of such protein may be obtained by expression of a full-length polynucleotide in a suitable mammalian cell or other host cell. The sequence of the mature form of the protein is also determinable from the amino acid sequence of the full-length form. Where proteins of the present invention are membrane bound, soluble forms of the proteins are also provided. In such forms, part or all of the regions causing the proteins to be membrane bound are deleted so that the proteins are fully secreted from the cell in which they are expressed.

Protein compositions of the present invention may further comprise an acceptable carrier, such as a hydrophilic, *e.g.*, pharmaceutically acceptable, carrier.

The present invention further provides isolated polypeptides encoded by the nucleic acid fragments of the present invention or by degenerate variants of the nucleic acid fragments of the present invention. By "degenerate variant" is intended nucleotide fragments which differ from a nucleic acid fragment of the present invention (*e.g.*, an ORF) by nucleotide sequence but, due to the degeneracy of the genetic code, encode an identical polypeptide sequence. Preferred nucleic acid fragments of the present invention are the ORFs that encode proteins.

A variety of methodologies known in the art can be utilized to obtain any one of the isolated polypeptides or proteins of the present invention. At the simplest level, the amino acid sequence can be synthesized using commercially available peptide synthesizers. The synthetically-constructed protein sequences, by virtue of sharing primary, secondary or tertiary structural and/or conformational characteristics with proteins may possess biological properties in common therewith, including protein activity. This technique is particularly useful in producing small peptides and fragments of larger polypeptides. Fragments are useful, for example, in generating antibodies against the native polypeptide. Thus, they may be employed as biologically active or immunological substitutes for natural, purified proteins in screening of therapeutic compounds and in immunological processes for the development of antibodies.

The polypeptides and proteins of the present invention can alternatively be purified from cells which have been altered to express the desired polypeptide or protein. As used herein, a cell is said to be altered to express a desired polypeptide or protein when the cell, through genetic manipulation, is made to produce a polypeptide or protein which it normally does not produce or which the cell normally produces at a lower level. One skilled in the art can readily adapt procedures for introducing and expressing either recombinant or synthetic sequences into eukaryotic or prokaryotic cells in order to generate a cell which produces one of the polypeptides or proteins of the present invention.

The invention also relates to methods for producing a polypeptide comprising growing a culture of host cells of the invention in a suitable culture medium, and purifying the protein from the cells or the culture in which the cells are grown. For example, the methods of the invention include a process for producing a polypeptide in which a host cell containing a suitable expression vector that includes a polynucleotide of the invention is cultured under conditions that allow expression of the encoded polypeptide. The polypeptide can be recovered from the culture, conveniently from the culture medium, or from a lysate prepared from the host cells and further purified. Preferred embodiments include those in which the protein produced by such process is a full length or mature form of the protein.

In an alternative method, the polypeptide or protein is purified from bacterial cells which naturally produce the polypeptide or protein. One skilled in the art can readily follow known methods for isolating polypeptides and proteins in order to obtain one of the isolated polypeptides or proteins of the present invention. These include, but are not limited to, immunochromatography, HPLC, size-exclusion chromatography, ion-exchange chromatography, and immuno-affinity chromatography. See, e.g., Scopes, *Protein Purification: Principles and Practice*, Springer-Verlag (1994); Sambrook, et al., in *Molecular Cloning: A Laboratory Manual*; Ausubel et al., *Current Protocols in Molecular Biology*. Polypeptide fragments that

retain biological/immunological activity include fragments comprising greater than about 100 amino acids, or greater than about 200 amino acids, and fragments that encode specific protein domains.

The purified polypeptides can be used in *in vitro* binding assays which are well known in the art to identify molecules which bind to the polypeptides. These molecules include but are not limited to, for *e.g.*, small molecules, molecules from combinatorial libraries, antibodies or other proteins. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

In addition, the peptides of the invention or molecules capable of binding to the peptides may be complexed with toxins, *e.g.*, ricin or cholera, or with other compounds that are toxic to cells. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for SEQ ID NO: 30369-60736.

The protein of the invention may also be expressed as a product of transgenic animals, *e.g.*, as a component of the milk of transgenic cows, goats, pigs, or sheep which are characterized by somatic or germ cells containing a nucleotide sequence encoding the protein.

The proteins provided herein also include proteins characterized by amino acid sequences similar to those of purified proteins but into which modification are naturally provided or deliberately engineered. For example, modifications in the peptide or DNA sequence can be made by those skilled in the art using known techniques. Modifications of interest in the protein sequences may include the alteration, substitution, replacement, insertion or deletion of a selected amino acid residue in the coding sequence. For example, one or more of the cysteine residues may be deleted or replaced with another amino acid to alter the conformation of the molecule. Techniques for such alteration, substitution, replacement, insertion or deletion are well known to those skilled in the art (see, *e.g.*, U.S. Pat. No. 4,518,584). Preferably, such alteration, substitution, replacement, insertion or deletion retains the desired activity of the protein. Regions of the protein that are important for the protein function can be determined by various methods known in the art including the alanine-scanning method which involved systematic substitution of single or strings of amino acids with alanine, followed by testing the resulting alanine-containing variant for biological activity. This type of analysis determines the importance of the substituted amino acid(s) in biological activity. Regions of the protein that are important for protein function may be determined by the eMATRIX program.

Other fragments and derivatives of the sequences of proteins which would be expected to retain protein activity in whole or in part and are useful for screening or other immunological

methodologies may also be easily made by those skilled in the art given the disclosures herein. Such modifications are encompassed by the present invention.

The protein may also be produced by operably linking the isolated polynucleotide of the invention to suitable control sequences in one or more insect expression vectors, and employing an insect expression system. Materials and methods for baculovirus/insect cell expression systems are commercially available in kit form from, *e.g.*, Invitrogen, San Diego, Calif., U.S.A. (the MaxBat™ kit), and such methods are well known in the art, as described in Summers and Smith, Texas Agricultural Experiment Station Bulletin No. 1555 (1987), incorporated herein by reference. As used herein, an insect cell capable of expressing a polynucleotide of the present invention is "transformed."

The protein of the invention may be prepared by culturing transformed host cells under culture conditions suitable to express the recombinant protein. The resulting expressed protein may then be purified from such culture (*i.e.*, from culture medium or cell extracts) using known purification processes, such as gel filtration and ion exchange chromatography. The purification of the protein may also include an affinity column containing agents which will bind to the protein; one or more column steps over such affinity resins as concanavalin A-agarose, heparin-toyopearl™ or Cibacrom blue 3GA Sepharose™; one or more steps involving hydrophobic interaction chromatography using such resins as phenyl ether, butyl ether, or propyl ether; or immunoaffinity chromatography.

Alternatively, the protein of the invention may also be expressed in a form that will facilitate purification. For example, it may be expressed as a fusion protein, such as those of maltose binding protein (MBP), glutathione-S-transferase (GST) or thioredoxin (TRX), or as a His-tag. Kits for expression and purification of such fusion proteins are commercially available from New England BioLab (Beverly, Mass.), Pharmacia (Piscataway, N.J.) and Invitrogen, respectively. The protein can also be tagged with an epitope and subsequently purified by using a specific antibody directed to such epitope. One such epitope ("FLAG®") is commercially available from Kodak (New Haven, Conn.).

Finally, one or more reverse-phase high performance liquid chromatography (RP- HPLC) steps employing hydrophobic RP-HPLC media, *e.g.*, silica gel having pendant methyl or other aliphatic groups, can be employed to further purify the protein. Some or all of the foregoing purification steps, in various combinations, can also be employed to provide a substantially homogeneous isolated recombinant protein. The protein thus purified is substantially free of other mammalian proteins and is defined in accordance with the present invention as an "isolated protein."

The polypeptides of the invention include analogs (variants). This embraces fragments, as well as peptides in which one or more amino acids has been deleted, inserted, or substituted. Also, analogs of the polypeptides of the invention embrace fusions of the polypeptides or modifications of the polypeptides of the invention, wherein the polypeptide or analog is fused to another moiety or moieties, *e.g.*, targeting moiety or another therapeutic agent. Such analogs may exhibit improved properties such as activity and/or stability. Examples of moieties which may be fused to the polypeptide or an analog include, for example, targeting moieties which provide for the delivery of polypeptide to pancreatic cells, *e.g.*, antibodies to pancreatic cells, antibodies to immune cells such as T-cells, monocytes, dendritic cells, granulocytes, etc., as well as receptor and ligands expressed on pancreatic or immune cells. Other moieties which may be fused to the polypeptide include therapeutic agents which are used for treatment, for example, immunosuppressive drugs such as cyclosporin, SK506, azathioprine, CD3 antibodies and steroids. Also, polypeptides may be fused to immune modulators, and other cytokines such as alpha or beta interferon.

4.6.1 DETERMINING POLYPEPTIDE AND POLYNUCLEOTIDE IDENTITY AND SIMILARITY

Preferred identity and/or similarity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in computer programs including, but are not limited to, the GCG program package, including GAP (Devereux, J., et al., Nucleic Acids Research 12(1):387 (1984); Genetics Computer Group, University of Wisconsin, Madison, WI), BLASTP, BLASTN, BLASTX, FASTA (Altschul, S.F. et al., J. Molec. Biol. 215:403-410 (1990), PSI-BLAST (Altschul S.F. et al., Nucleic Acids Res. vol. 25, pp. 3389-3402, herein incorporated by reference), eMatrix software (Wu et al., J. Comp. Biol., Vol. 6, pp. 219-235 (1999), herein incorporated by reference), eMotif software (Nevill-Manning et al, ISMB-97, Vol. 4, pp. 202-209, herein incorporated by reference), pFam software (Sonnhammer et al., Nucleic Acids Res., Vol. 26(1), pp. 320-322 (1998), herein incorporated by reference) and the Kyte-Doolittle hydrophobicity prediction algorithm (J. Mol Biol, 157, pp. 105-31 (1982), incorporated herein by reference). The BLAST programs are publicly available from the National Center for Biotechnology Information (NCBI) and other sources (BLAST Manual, Altschul, S., et al. NCB NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990).

4.7 CHIMERIC AND FUSION PROTEINS

The invention also provides chimeric or fusion proteins. As used herein, a "chimeric protein" or "fusion protein" comprises a polypeptide of the invention operatively linked to

another polypeptide. Within a fusion protein the polypeptide according to the invention can correspond to all or a portion of a protein according to the invention. In one embodiment, a fusion protein comprises at least one biologically active portion of a protein according to the invention. In another embodiment, a fusion protein comprises at least two biologically active portions of a protein according to the invention. Within the fusion protein, the term "operatively linked" is intended to indicate that the polypeptide according to the invention and the other polypeptide are fused in-frame to each other. The polypeptide can be fused to the N-terminus or C-terminus.

For example, in one embodiment a fusion protein comprises a polypeptide according to the invention operably linked to the extracellular domain of a second protein.

In another embodiment, the fusion protein is a GST-fusion protein in which the polypeptide sequences of the invention are fused to the C-terminus of the GST (*i.e.*, glutathione S-transferase) sequences.

In another embodiment, the fusion protein is an immunoglobulin fusion protein in which the polypeptide sequences according to the invention comprises one or more domains are fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin fusion proteins of the invention can be incorporated into pharmaceutical compositions and administered to a subject to inhibit an interaction between a ligand and a protein of the invention on the surface of a cell, to thereby suppress signal transduction *in vivo*.

The immunoglobulin fusion proteins can be used to affect the bioavailability of a cognate ligand. Inhibition of the ligand/protein interaction may be useful therapeutically for both the treatment of proliferative and differentiative disorders, *e.g.*, cancer as well as modulating (*e.g.*, promoting or inhibiting) cell survival. Moreover, the immunoglobulin fusion proteins of the invention can be used as immunogens to produce antibodies in a subject, to purify ligands, and in screening assays to identify molecules that inhibit the interaction of a polypeptide of the invention with a ligand.

A chimeric or fusion protein of the invention can be produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, *e.g.*, by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining, and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers.

Alternatively, PCR amplification of gene fragments can be carried out using anchor primers that give rise to complementary overhangs between two consecutive gene fragments that can subsequently be annealed and reamplified to generate a chimeric gene sequence (see, for

example, Ausubel et al. (eds.) CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A nucleic acid encoding a polypeptide of the invention can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the protein of the invention.

4.8 GENE THERAPY

Mutations in the polynucleotides of the invention gene may result in loss of normal function of the encoded protein. The invention thus provides gene therapy to restore normal activity of the polypeptides of the invention; or to treat disease states involving polypeptides of the invention. Delivery of a functional gene encoding polypeptides of the invention to appropriate cells is effected *ex vivo*, *in situ*, or *in vivo* by use of vectors, and more particularly viral vectors (e.g., adenovirus, adeno-associated virus, or a retrovirus), or *ex vivo* by use of physical DNA transfer methods (e.g., liposomes or chemical treatments). See, for example, Anderson, Nature, supplement to vol. 392, no. 6679, pp.25-20 (1998). For additional reviews of gene therapy technology see Friedmann, Science, 244: 1275-1281 (1989); Verma, Scientific American: 68-84 (1990); and Miller, Nature, 357: 455-460 (1992). Introduction of any one of the nucleotides of the present invention or a gene encoding the polypeptides of the present invention can also be accomplished with extrachromosomal substrates (transient expression) or artificial chromosomes (stable expression). Cells may also be cultured *ex vivo* in the presence of proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced *in vivo* for therapeutic purposes. Alternatively, it is contemplated that in other human disease states, preventing the expression of or inhibiting the activity of polypeptides of the invention will be useful in treating the disease states. It is contemplated that antisense therapy or gene therapy could be applied to negatively regulate the expression of polypeptides of the invention.

Other methods inhibiting expression of a protein include the introduction of antisense molecules to the nucleic acids of the present invention, their complements, or their translated RNA sequences, by methods known in the art. Further, the polypeptides of the present invention can be inhibited by using targeted deletion methods, or the insertion of a negative regulatory element such as a silencer, which is tissue specific.

The present invention still further provides cells genetically engineered *in vivo* to express the polynucleotides of the invention, wherein such polynucleotides are in operative association with a regulatory sequence heterologous to the host cell which drives expression of the polynucleotides in

the cell. These methods can be used to increase or decrease the expression of the polynucleotides of the present invention.

Knowledge of DNA sequences provided by the invention allows for modification of cells to permit, increase, or decrease, expression of endogenous polypeptide. Cells can be modified (*e.g.*, by homologous recombination) to provide increased polypeptide expression by replacing, in whole or in part, the naturally occurring promoter with all or part of a heterologous promoter so that the cells express the protein at higher levels. The heterologous promoter is inserted in such a manner that it is operatively linked to the desired protein encoding sequences. See, for example, PCT International Publication No. WO 94/12650, PCT International Publication No. WO 92/20808, and PCT International Publication No. WO 91/09955. It is also contemplated that, in addition to heterologous promoter DNA, amplifiable marker DNA (*e.g.*, *ada*, *dhfr*, and the multifunctional CAD gene which encodes carbamyl phosphate synthase, aspartate transcarbamylase, and dihydroorotase) and/or intron DNA may be inserted along with the heterologous promoter DNA. If linked to the desired protein coding sequence, amplification of the marker DNA by standard selection methods results in co-amplification of the desired protein coding sequences in the cells.

In another embodiment of the present invention, cells and tissues may be engineered to express an endogenous gene comprising the polynucleotides of the invention under the control of inducible regulatory elements, in which case the regulatory sequences of the endogenous gene may be replaced by homologous recombination. As described herein, gene targeting can be used to replace a gene's existing regulatory region with a regulatory sequence isolated from a different gene or a novel regulatory sequence synthesized by genetic engineering methods. Such regulatory sequences may be comprised of promoters, enhancers, scaffold-attachment regions, negative regulatory elements, transcriptional initiation sites, regulatory protein binding sites or combinations of said sequences. Alternatively, sequences which affect the structure or stability of the RNA or protein produced may be replaced, removed, added, or otherwise modified by targeting. These sequences include polyadenylation signals, mRNA stability elements, splice sites, leader sequences for enhancing or modifying transport or secretion properties of the protein, or other sequences which alter or improve the function or stability of protein or RNA molecules.

The targeting event may be a simple insertion of the regulatory sequence, placing the gene under the control of the new regulatory sequence, *e.g.*, inserting a new promoter or enhancer or both upstream of a gene. Alternatively, the targeting event may be a simple deletion of a regulatory element, such as the deletion of a tissue-specific negative regulatory element. Alternatively, the targeting event may replace an existing element; for example, a tissue-specific enhancer can be replaced by an enhancer that has broader or different cell-type specificity than the naturally occurring elements. Here, the naturally occurring sequences are deleted and new sequences are

added. In all cases, the identification of the targeting event may be facilitated by the use of one or more selectable marker genes that are contiguous with the targeting DNA, allowing for the selection of cells in which the exogenous DNA has integrated into the cell genome. The identification of the targeting event may also be facilitated by the use of one or more marker genes exhibiting the property of negative selection, such that the negatively selectable marker is linked to the exogenous DNA, but configured such that the negatively selectable marker flanks the targeting sequence, and such that a correct homologous recombination event with sequences in the host cell genome does not result in the stable integration of the negatively selectable marker. Markers useful for this purpose include the Herpes Simplex Virus thymidine kinase (TK) gene or the bacterial xanthine-guanine phosphoribosyl-transferase (gpt) gene.

The gene targeting or gene activation techniques which can be used in accordance with this aspect of the invention are more particularly described in U.S. Patent No. 5,272,071 to Chappel; U.S. Patent No. 5,578,461 to Sherwin et al.; International Application No. PCT/US92/09627 (WO93/09222) by Selden et al.; and International Application No. PCT/US90/06436 (WO91/06667) by Skoultschi et al., each of which is incorporated by reference herein in its entirety.

4.9 TRANSGENIC ANIMALS

In preferred methods to determine biological functions of the polypeptides of the invention in vivo, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of a promoter of the polynucleotides of the invention is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The

homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

The polynucleotides of the present invention also make possible the development, through, *e.g.*, homologous recombination or knock out strategies, of animals that fail to express polypeptides of the invention or that express a variant polypeptide. Such animals are useful as models for studying the *in vivo* activities of polypeptide as well as for studying modulators of the polypeptides of the invention.

In preferred methods to determine biological functions of the polypeptides of the invention *in vivo*, one or more genes provided by the invention are either over expressed or inactivated in the germ line of animals using homologous recombination [Capecchi, Science 244:1288-1292 (1989)]. Animals in which the gene is over expressed, under the regulatory control of exogenous or endogenous promoter elements, are known as transgenic animals. Animals in which an endogenous gene has been inactivated by homologous recombination are referred to as "knockout" animals. Knockout animals, preferably non-human mammals, can be prepared as described in U.S. Patent No. 5,557,032, incorporated herein by reference. Transgenic animals are useful to determine the roles polypeptides of the invention play in biological processes, and preferably in disease states. Transgenic animals are useful as model systems to identify compounds that modulate lipid metabolism. Transgenic animals, preferably non-human mammals, are produced using methods as described in U.S. Patent No 5,489,743 and PCT Publication No. WO94/28122, incorporated herein by reference.

Transgenic animals can be prepared wherein all or part of the polynucleotides of the invention promoter is either activated or inactivated to alter the level of expression of the polypeptides of the invention. Inactivation can be carried out using homologous recombination methods described above. Activation can be achieved by supplementing or even replacing the homologous promoter to provide for increased protein expression. The homologous promoter can be supplemented by insertion of one or more heterologous enhancer elements known to confer promoter activation in a particular tissue.

4.10 USES AND BIOLOGICAL ACTIVITY

The polynucleotides and proteins of the present invention are expected to exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified herein. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA). The mechanism underlying the particular condition or pathology will dictate whether the

polypeptides of the invention, the polynucleotides of the invention or modulators (activators or inhibitors) thereof would be beneficial to the subject in need of treatment. Thus, "therapeutic compositions of the invention" include compositions comprising isolated polynucleotides (including recombinant DNA molecules, cloned genes and degenerate variants thereof) or polypeptides of the invention (including full length protein, mature protein and truncations or domains thereof), or compounds and other substances that modulate the overall activity of the target gene products, either at the level of target gene/protein expression or target protein activity. Such modulators include polypeptides, analogs, (variants), including fragments and fusion proteins, antibodies and other binding proteins; chemical compounds that directly or indirectly activate or inhibit the polypeptides of the invention (identified, *e.g.*, via drug screening assays as described herein); antisense polynucleotides and polynucleotides suitable for triple helix formation; and in particular antibodies or other binding partners that specifically recognize one or more epitopes of the polypeptides of the invention.

The polypeptides of the present invention may likewise be involved in cellular activation or in one of the other physiological pathways described herein.

4.10.1 RESEARCH USES AND UTILITIES

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on gels; as chromosome markers or tags (when labeled) to identify chromosomes or to map related gene positions; to compare with endogenous DNA sequences in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for genetic fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene chip" or other support, including for examination of expression patterns; to raise anti-protein antibodies using DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The polypeptides provided by the present invention can similarly be used in assays to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding polypeptide is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E. F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S. L. and A. R. Kimmel eds., 1987.

4.10.2 NUTRITIONAL USES

Polynucleotides and polypeptides of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the polypeptide or polynucleotide of the invention can be added to the feed of a particular organism or can be administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the polypeptide or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

4.10.3 CYTOKINE AND CELL PROLIFERATION/DIFFERENTIATION ACTIVITY

A polypeptide of the present invention may exhibit activity relating to cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor-dependent cell proliferation assays, and hence the assays serve as a convenient

confirmation of cytokine activity. The activity of therapeutic compositions of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+(preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e, CMK,

5 HUVEC, and Caco. Therapeutic compositions of the invention can be used in the following:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in
10 Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Bertagnolli et al., J. Immunol. 145:1706-1712, 1990; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Bertagnolli, et al., I. Immunol. 149:3778-3783, 1992; Bowman et al., I. Immunol. 152:1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation,
15 Kruisbeek, A. M. and Shevach, E. M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human interleukin- γ , Schreiber, R. D. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of hematopoietic and lymphopoietic cells
20 include, without limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L. S. and Lipsky, P. E. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991; Moreau et al., Nature 336:690-692, 1988; Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse
25 and human interleukin 6--Nordan, R. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11--Bennett, F., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin
30 9--Ciarletta, A., Giannotti, J., Clark, S. C. and Turner, K. J. In Current Protocols in Immunology. J. E. Coligan eds. Vol 1 pp. 6.13.1, John Wiley and Sons, Toronto. 1991.

Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without limitation, those described in: Current Protocols in
35 Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W Strober,

Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, *In Vitro* assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. USA 77:6091-6095, 1980; Weinberger et al., Eur. J. Immun. 11:405-411, 1981; Takai et al., J. Immunol.

5 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

4.10.4 STEM CELL GROWTH FACTOR ACTIVITY

A polypeptide of the present invention may exhibit stem cell growth factor activity and be involved in the proliferation, differentiation and survival of pluripotent and totipotent stem cells including primordial germ cells, embryonic stem cells, hematopoietic stem cells and/or germ line stem cells. Administration of the polypeptide of the invention to stem cells *in vivo* or *ex vivo* is expected to maintain and expand cell populations in a totipotent or pluripotent state which would be useful for re-engineering damaged or diseased tissues, transplantation, manufacture of bio-pharmaceuticals and the development of bio-sensors. The ability to produce large quantities of human cells has important working applications for the production of human proteins which currently must be obtained from non-human sources or donors, implantation of cells to treat diseases such as Parkinson's, Alzheimer's and other neurodegenerative diseases; tissues for grafting such as bone marrow, skin, cartilage, tendons, bone, muscle (including cardiac muscle), blood vessels, cornea, neural cells, gastrointestinal cells and others; and organs for transplantation such as kidney, liver, pancreas (including islet cells), heart and lung.

It is contemplated that multiple different exogenous growth factors and/or cytokines may be administered in combination with the polypeptide of the invention to achieve the desired effect, including any of the growth factors listed herein, other stem cell maintenance factors, and specifically including stem cell factor (SCF), leukemia inhibitory factor (LIF), Flt-3 ligand (Flt-3L), any of the interleukins, recombinant soluble IL-6 receptor fused to IL-6, macrophage inflammatory protein 1-alpha (MIP-1-alpha), G-CSF, GM-CSF, thrombopoietin (TPO), platelet factor 4 (PF-4), platelet-derived growth factor (PDGF), neural growth factors and basic fibroblast growth factor (bFGF).

Since totipotent stem cells can give rise to virtually any mature cell type, expansion of these cells in culture will facilitate the production of large quantities of mature cells. Techniques for culturing stem cells are known in the art and administration of polypeptides of the invention, optionally with other growth factors and/or cytokines, is expected to enhance the survival and proliferation of the stem cell populations. This can be accomplished by direct administration of the polypeptide of the invention to the culture medium. Alternatively, stroma cells transfected with a polynucleotide that encodes for the polypeptide of the invention can be used as a feeder

layer for the stem cell populations in culture or in vivo. Stromal support cells for feeder layers may include embryonic bone marrow fibroblasts, bone marrow stromal cells, fetal liver cells, or cultured embryonic fibroblasts (see U.S. Patent No. 5,690,926).

Stem cells themselves can be transfected with a polynucleotide of the invention to induce autocrine expression of the polypeptide of the invention. This will allow for generation of undifferentiated totipotent/pluripotent stem cell lines that are useful as is or that can then be differentiated into the desired mature cell types. These stable cell lines can also serve as a source of undifferentiated totipotent/pluripotent mRNA to create cDNA libraries and templates for polymerase chain reaction experiments. These studies would allow for the isolation and identification of differentially expressed genes in stem cell populations that regulate stem cell proliferation and/or maintenance.

Expansion and maintenance of totipotent stem cell populations will be useful in the treatment of many pathological conditions. For example, polypeptides of the present invention may be used to manipulate stem cells in culture to give rise to neuroepithelial cells that can be used to augment or replace cells damaged by illness, autoimmune disease, accidental damage or genetic disorders. The polypeptide of the invention may be useful for inducing the proliferation of neural cells and for the regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders which involve degeneration, death or trauma to neural cells or nerve tissue. In addition, the expanded stem cell populations can also be genetically altered for gene therapy purposes and to decrease host rejection of replacement tissues after grafting or implantation.

Expression of the polypeptide of the invention and its effect on stem cells can also be manipulated to achieve controlled differentiation of the stem cells into more differentiated cell types. A broadly applicable method of obtaining pure populations of a specific differentiated cell type from undifferentiated stem cell populations involves the use of a cell-type specific promoter driving a selectable marker. The selectable marker allows only cells of the desired type to survive. For example, stem cells can be induced to differentiate into cardiomyocytes (Wobus et al., *Differentiation*, 48: 173-182, (1991); Klug et al., *J. Clin. Invest.*, 98(1): 216-224, (1998)) or skeletal muscle cells (Browder, L. W. In: *Principles of Tissue Engineering* eds. Lanza et al., Academic Press (1997)). Alternatively, directed differentiation of stem cells can be accomplished by culturing the stem cells in the presence of a differentiation factor such as retinoic acid and an antagonist of the polypeptide of the invention which would inhibit the effects of endogenous stem cell factor activity and allow differentiation to proceed.

In vitro cultures of stem cells can be used to determine if the polypeptide of the invention exhibits stem cell growth factor activity. Stem cells are isolated from any one of various cell

sources (including hematopoietic stem cells and embryonic stem cells) and cultured on a feeder layer, as described by Thompson et al. Proc. Natl. Acad. Sci, U.S.A., 92: 7844-7848 (1995), in the presence of the polypeptide of the invention alone or in combination with other growth factors or cytokines. The ability of the polypeptide of the invention to induce stem cells proliferation is determined by colony formation on semi-solid support *e.g.* as described by Bernstein et al., Blood, 77: 2316-2321 (1991).

4.10.5 HEMATOPOIESIS REGULATING ACTIVITY

A polypeptide of the present invention may be involved in regulation of hematopoiesis and, consequently, in the treatment of myeloid or lymphoid cell disorders. Even marginal biological activity in support of colony forming cells or of factor-dependent cell lines indicates involvement in regulating hematopoiesis, *e.g.* in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation of myeloid cells such as granulocytes and monocytes/macrophages (*i.e.*, traditional CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting the growth and proliferation of megakaryocytes and consequently of platelets thereby allowing prevention or treatment of various platelet disorders such as thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the above-mentioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without limitation, aplastic anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either *in-vivo* or *ex-vivo* (*i.e.*, in conjunction with bone marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

Therapeutic compositions of the invention can be used in the following:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming assays, Freshney, M. G. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, N.Y. 1994; Hirayama et al., Proc. Natl. Acad. Sci. USA 89:5907-5911, 1992; Primitive hematopoietic colony forming cells with high proliferative potential, McNiece, I. K. and Briddell, R. A. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, N.Y. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R. E. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, N.Y. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, N.Y. 1994; Long term culture initiating cell assay, Sutherland, H. J. In Culture of Hematopoietic Cells. R. I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, N.Y. 1994.

4.10.6 TISSUE GROWTH ACTIVITY

A polypeptide of the present invention also may be involved in bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as in wound healing and tissue repair and replacement, and in healing of burns, incisions and ulcers.

A polypeptide of the present invention which induces cartilage and/or bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Compositions of a polypeptide, antibody, binding partner, or other modulator of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

A polypeptide of this invention may also be involved in attracting bone-forming cells, stimulating growth of bone-forming cells, or inducing differentiation of progenitors of bone-forming cells. Treatment of osteoporosis, osteoarthritis, bone degenerative disorders, or periodontal disease, such as through stimulation of bone and/or cartilage repair or by blocking inflammation or processes of tissue destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes may also be possible using the composition of the invention.

Another category of tissue regeneration activity that may involve the polypeptide of the present invention is tendon/ligament formation. Induction of tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by a composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide environment to attract tendon- or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendon- or ligament-forming cells, or induce growth of tendon/ligament cells or progenitors *ex vivo* for return *in vivo* to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. The compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

The compositions of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, *i.e.* for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve tissue. More specifically, a composition may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions that may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a composition of the invention.

Compositions of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

Compositions of the present invention may also be involved in the generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine,

kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring may allow normal tissue to regenerate. A polypeptide of the present invention may also exhibit angiogenic activity.

5 A composition of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A composition of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the
10 growth of tissues described above.

Therapeutic compositions of the invention can be used in the following:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. WO95/16035 (bone, cartilage, tendon); International Patent Publication No. WO95/05846 (nerve, neuronal); International Patent Publication No.
15 WO91/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, H. I. and Rovee, D. T., eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

4.10.7 IMMUNE STIMULATING OR SUPPRESSING ACTIVITY

A polypeptide of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A polynucleotide of the invention can encode a polypeptide exhibiting such activities. A
25 protein may be useful in the treatment of various immune deficiencies and disorders (including severe combined immunodeficiency (SCID)), *e.g.*, in regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These immune deficiencies may be genetic or be caused by viral (*e.g.*, HIV) as well as bacterial or fungal infections, or may result from autoimmune disorders. More
30 specifically, infectious diseases caused by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpes viruses, mycobacteria, *Leishmania* spp., malaria spp. and various fungal infections such as candidiasis. Of course, in this regard, proteins of the present invention may also be useful where a boost to the immune system generally may be desirable, *i.e.*, in the treatment of cancer.

Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic lupus erythematosus, rheumatoid arthritis, autoimmune pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitus, myasthenia gravis, graft-versus-host disease and autoimmune inflammatory eye disease. Such a protein (or antagonists thereof, including antibodies) of the present invention may also be useful in the treatment of allergic reactions and conditions (*e.g.*, anaphylaxis, serum sickness, drug reactions, food allergies, insect venom allergies, mastocytosis, allergic rhinitis, hypersensitivity pneumonitis, urticaria, angioedema, eczema, atopic dermatitis, allergic contact dermatitis, erythema multiforme, Stevens-Johnson syndrome, allergic conjunctivitis, atopic keratoconjunctivitis, venereal keratoconjunctivitis, giant papillary conjunctivitis and contact allergies), such as asthma (particularly allergic asthma) or other respiratory problems. Other conditions, in which immune suppression is desired (including, for example, organ transplantation), may also be treatable using a protein (or antagonists thereof) of the present invention. The therapeutic effects of the polypeptides or antagonists thereof on allergic reactions can be evaluated by *in vivo* animals models such as the cumulative contact enhancement test (Lastbom et al., Toxicology 125: 59-66, 1998), skin prick test (Hoffmann et al., Allergy 54: 446-54, 1999), guinea pig skin sensitization test (Vohr et al., Arch. Toxicol. 73: 501-9), and murine local lymph node assay (Kimber et al., J. Toxicol. Environ. Health 53: 563-79).

Using the proteins of the invention it may also be possible to modulate immune responses, in a number of ways. Down regulation may be in the form of inhibiting or blocking an immune response already in progress or may involve preventing the induction of an immune response. The functions of activated T cells may be inhibited by suppressing T cell responses or by inducing specific tolerance in T cells, or both. Immunosuppression of T cell responses is generally an active, non-antigen-specific, process which requires continuous exposure of the T cells to the suppressive agent. Tolerance, which involves inducing non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigen-specific and persists after exposure to the tolerizing agent has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as, for example, B7)), *e.g.*, preventing high level lymphokine synthesis by activated T cells, will be useful in situations of tissue, skin and organ transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in reduced tissue destruction in tissue transplantation. Typically, in tissue

transplants, rejection of the transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a therapeutic composition of the invention may prevent cytokine synthesis by immune cells, such as T cells, and thus acts as an immunosuppressant. Moreover, a lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by B lymphocyte antigen-blocking reagents may avoid the necessity of repeated administration of these blocking reagents. To achieve sufficient immunosuppression or tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

The efficacy of particular therapeutic compositions in preventing organ transplant rejection or GVHD can be assessed using animal models that are predictive of efficacy in humans. Examples of appropriate systems which can be used include allogeneic cardiac grafts in rats and xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Ig fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). In addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of therapeutic compositions of the invention on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self-tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate disease symptoms. Administration of reagents which block stimulation of T cells can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. The efficacy of blocking reagents in preventing or alleviating autoimmune disorders can be determined using a number of well-characterized animal models of human autoimmune diseases. Examples include murine experimental autoimmune encephalitis, systemic lupus erythematosus in MRL/lpr/lpr mice or NZB hybrid mice, murine autoimmune collagen arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

Upregulation of an antigen function (*e.g.*, a B lymphocyte antigen function), as a means of up regulating immune responses, may also be useful in therapy. Upregulation of immune

responses may be in the form of enhancing an existing immune response or eliciting an initial immune response. For example, enhancing an immune response may be useful in cases of viral infection, including systemic viral diseases such as influenza, the common cold, and encephalitis.

5 Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected
10 cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

A polypeptide of the present invention may provide the necessary stimulation signal to T
15 cells to induce a T cell mediated immune response against the transfected tumor cells. In addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient mounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (*e.g.*, a cytoplasmic-domain truncated portion) of an MHC class I alpha chain protein and β_2 microglobulin protein or an MHC class II alpha chain
20 protein and an MHC class II beta chain protein to thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (*e.g.*, B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as
25 the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific immunity. Thus, the induction of a T cell mediated immune response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

The activity of a protein of the invention may, among other means, be measured by the
30 following methods:

Suitable assays for thymocyte or splenocyte cytotoxicity include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19;
35 Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA

78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bowman et al., J. Virology 61:1992-1998; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

5 Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J. J. and Brunswick, M. In Current Protocols in Immunology. J. E. e.a. Coligan eds. Vol 1
10 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

 Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3,
15 In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

 Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in:
20 Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965, 1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation
25 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

 Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research
30 53:1945-1951, 1993; Itoh et al., Cell 66:233-243, 1991; Zacharchuk, Journal of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

 Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et

al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad. Sci. USA 88:7548-7551, 1991.

4.10.8 ACTIVIN/INHIBIN ACTIVITY

5 A polypeptide of the present invention may also exhibit activin- or inhibin-related activities. A polynucleotide of the invention may encode a polypeptide exhibiting such characteristics. Inhibins are characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a polypeptide of the present invention,
10 alone or in heterodimers with a member of the inhibin family, may be useful as a contraceptive based on the ability of inhibins to decrease fertility in female mammals and decrease spermatogenesis in male mammals. Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the polypeptide of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin group, may be useful as
15 a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, U.S. Pat. No. 4,798,885. A polypeptide of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as, but not limited to, cows, sheep and pigs.

20 The activity of a polypeptide of the invention may, among other means, be measured by the following methods.

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci.
25 USA 83:3091-3095, 1986.

4.10.9 CHEMOTACTIC/CHEMOKINETIC ACTIVITY

A polypeptide of the present invention may be involved in chemotactic or chemokinetic activity for mammalian cells, including, for example, monocytes, fibroblasts, neutrophils,
30 T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Chemotactic and chemokinetic receptor activation can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic compositions (*e.g.* proteins, antibodies, binding partners, or modulators of the invention) provide particular advantages in treatment of wounds and other
35 trauma to tissues, as well as in treatment of localized infections. For example, attraction of

lymphocytes, monocytes or neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population.

- 5 Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

Therapeutic compositions of the invention can be used in the following:

- 10 Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Marguiles, E. M. Shevach, W. Strober; Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines
15 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25:1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153:1762-1768, 1994.

20 **4.10.10 HEMOSTATIC AND THROMBOLYTIC ACTIVITY**

- A polypeptide of the invention may also be involved in hemostasis or thrombolysis or thrombosis. A polynucleotide of the invention can encode a polypeptide exhibiting such attributes. Compositions may be useful in treatment of various coagulation disorders (including hereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events
25 in treating wounds resulting from trauma, surgery or other causes. A composition of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (*e.g.*, stroke).

Therapeutic compositions of the invention can be used in the following:

- 30 Assay for hemostatic and thrombolytic activity include, without limitation, those described in: Linet et al., J. Clin. Pharmacol. 26:131-140, 1986; Burdick et al., Thrombosis Res. 45:413-419, 1987; Humphrey et al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474, 1988.

35 **4.10.11 CANCER DIAGNOSIS AND THERAPY**

Polypeptides of the invention may be involved in cancer cell generation, proliferation or metastasis. Detection of the presence or amount of polynucleotides or polypeptides of the invention may be useful for the diagnosis and/or prognosis of one or more types of cancer. For example, the presence or increased expression of a polynucleotide/polypeptide of the invention may indicate a hereditary risk of cancer, a precancerous condition, or an ongoing malignancy. Conversely, a defect in the gene or absence of the polypeptide may be associated with a cancer condition. Identification of single nucleotide polymorphisms associated with cancer or a predisposition to cancer may also be useful for diagnosis or prognosis.

Cancer treatments promote tumor regression by inhibiting tumor cell proliferation, inhibiting angiogenesis (growth of new blood vessels that is necessary to support tumor growth) and/or prohibiting metastasis by reducing tumor cell motility or invasiveness. Therapeutic compositions of the invention may be effective in adult and pediatric oncology including in solid phase tumors/malignancies, locally advanced tumors, human soft tissue sarcomas, metastatic cancer, including lymphatic metastases, blood cell malignancies including multiple myeloma, acute and chronic leukemias, and lymphomas, head and neck cancers including mouth cancer, larynx cancer and thyroid cancer, lung cancers including small cell carcinoma and non-small cell cancers, breast cancers including small cell carcinoma and ductal carcinoma, gastrointestinal cancers including esophageal cancer, stomach cancer, colon cancer, colorectal cancer and polyps associated with colorectal neoplasia, pancreatic cancers, liver cancer, urologic cancers including bladder cancer and prostate cancer, malignancies of the female genital tract including ovarian carcinoma, uterine (including endometrial) cancers, and solid tumor in the ovarian follicle, kidney cancers including renal cell carcinoma, brain cancers including intrinsic brain tumors, neuroblastoma, astrocytic brain tumors, gliomas, metastatic tumor cell invasion in the central nervous system, bone cancers including osteomas, skin cancers including malignant melanoma, tumor progression of human skin keratinocytes, squamous cell carcinoma, basal cell carcinoma, hemangiopericytoma and Kaposi's sarcoma.

Polypeptides, polynucleotides, or modulators of polypeptides of the invention (including inhibitors and stimulators of the biological activity of the polypeptide of the invention) may be administered to treat cancer. Therapeutic compositions can be administered in therapeutically effective dosages alone or in combination with adjuvant cancer therapy such as surgery, chemotherapy, radiotherapy, thermotherapy, and laser therapy, and may provide a beneficial effect, *e.g.* reducing tumor size, slowing rate of tumor growth, inhibiting metastasis, or otherwise improving overall clinical condition, without necessarily eradicating the cancer.

The composition can also be administered in therapeutically effective amounts as a portion of an anti-cancer cocktail. An anti-cancer cocktail is a mixture of the polypeptide or

modulator of the invention with one or more anti-cancer drugs in addition to a pharmaceutically acceptable carrier for delivery. The use of anti-cancer cocktails as a cancer treatment is routine. Anti-cancer drugs that are well known in the art and can be used as a treatment in combination with the polypeptide or modulator of the invention include: Actinomycin D,

5 Aminoglutethimide, Asparaginase, Bleomycin, Busulfan, Carboplatin, Carmustine, Chlorambucil, Cisplatin (cis-DDP), Cyclophosphamide, Cytarabine HCl (Cytosine arabinoside), Dacarbazine, Dactinomycin, Daunorubicin HCl, Doxorubicin HCl, Estramustine phosphate sodium, Etoposide (V16-213), Floxuridine, 5-Fluorouracil (5-Fu), Flutamide, Hydroxyurea (hydroxycarbamide), Ifosfamide, Interferon Alpha-2a, Interferon Alpha-2b, Leuprolide acetate
10 (LHRH-releasing factor analog), Lomustine, Mechlorethamine HCl (nitrogen mustard), Melphalan, Mercaptopurine, Mesna, Methotrexate (MTX), Mitomycin, Mitoxantrone HCl, Octreotide, Plicamycin, Procarbazine HCl, Streptozocin, Tamoxifen citrate, Thioguanine, Thiotepa, Vinblastine sulfate, Vincristine sulfate, Amsacrine, Azacitidine, Hexamethylmelamine, Interleukin-2, Mitoguazone, Pentostatin, Semustine, Teniposide, and Vindesine sulfate.

15 In addition, therapeutic compositions of the invention may be used for prophylactic treatment of cancer. There are hereditary conditions and/or environmental situations (*e.g.* exposure to carcinogens) known in the art that predispose an individual to developing cancers. Under these circumstances, it may be beneficial to treat these individuals with therapeutically effective doses of the polypeptide of the invention to reduce the risk of developing cancers.

20 *In vitro* models can be used to determine the effective doses of the polypeptide of the invention as a potential cancer treatment. These *in vitro* models include proliferation assays of cultured tumor cells, growth of cultured tumor cells in soft agar (see Freshney, (1987) Culture of Animal Cells: A Manual of Basic Technique, Wiley-Liss, New York, NY Ch 18 and Ch 21), tumor systems in nude mice as described in Giovanella et al., J. Natl. Can. Inst., 52: 921-30
25 (1974), mobility and invasive potential of tumor cells in Boyden Chamber assays as described in Pilkington et al., Anticancer Res., 17: 4107-9 (1997), and angiogenesis assays such as induction of vascularization of the chick chorioallantoic membrane or induction of vascular endothelial cell migration as described in Ribatta et al., Intl. J. Dev. Biol., 40: 1189-97 (1999) and Li et al., Clin. Exp. Metastasis, 17:423-9 (1999), respectively. Suitable tumor cells lines are available,
30 *e.g.* from American Type Tissue Culture Collection catalogs.

4.10.12 RECEPTOR/LIGAND ACTIVITY

A polypeptide of the present invention may also demonstrate activity as receptor, receptor ligand or inhibitor or agonist of receptor/ligand interactions. A polynucleotide of the
35 invention can encode a polypeptide exhibiting such characteristics. Examples of such receptors

and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their ligands (including without limitation, cellular adhesion molecules (such as selectins, integrins and their ligands) and receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral immune responses. Receptors and ligands are also useful for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. A protein of the present invention (including, without limitation, fragments of receptors and ligands) may themselves be useful as inhibitors of receptor/ligand interactions.

The activity of a polypeptide of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, W. Strober, Pub. Greene Publishing Associates and Wiley- Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1- 7.28.22), Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

By way of example, the polypeptides of the invention may be used as a receptor for a ligand(s) thereby transmitting the biological activity of that ligand(s). Ligands may be identified through binding assays, affinity chromatography, dihybrid screening assays, BIAcore assays, gel overlay assays, or other methods known in the art.

Studies characterizing drugs or proteins as agonist or antagonist or partial agonists or a partial antagonist require the use of other proteins as competing ligands. The polypeptides of the present invention or ligand(s) thereof may be labeled by being coupled to radioisotopes, colorimetric molecules or toxin molecules by conventional methods. ("Guide to Protein Purification" Murray P. Deutscher (ed) Methods in Enzymology Vol. 182 (1990) Academic Press, Inc. San Diego). Examples of radioisotopes include, but are not limited to, tritium and carbon-14. Examples of colorimetric molecules include, but are not limited to, fluorescent molecules such as fluorescamine, or rhodamine or other colorimetric molecules. Examples of toxins include, but are not limited, to ricin.

4.10.13 DRUG SCREENING

This invention is particularly useful for screening chemical compounds by using the novel polypeptides or binding fragments thereof in any of a variety of drug screening techniques.

The polypeptides or fragments employed in such a test may either be free in solution, affixed to a solid support, borne on a cell surface or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or a fragment thereof. Drugs are screened against such transformed cells in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example, the formation of complexes between polypeptides of the invention or fragments and the agent being tested or examine the diminution in complex formation between the novel polypeptides and an appropriate cell line, which are well known in the art.

Sources for test compounds that may be screened for ability to bind to or modulate (*i.e.*, increase or decrease) the activity of polypeptides of the invention include (1) inorganic and organic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of either random or mimetic peptides, oligonucleotides or organic molecules.

Chemical libraries may be readily synthesized or purchased from a number of commercial sources, and may include structural analogs of known compounds or compounds that are identified as "hits" or "leads" via natural product screening.

The sources of natural product libraries are microorganisms (including bacteria and fungi), animals, plants or other vegetation, or marine organisms, and libraries of mixtures for screening may be created by: (1) fermentation and extraction of broths from soil, plant or marine microorganisms or (2) extraction of the organisms themselves. Natural product libraries include polyketides, non-ribosomal peptides, and (non-naturally occurring) variants thereof. For a review, see *Science* 282:63-68 (1998).

Combinatorial libraries are composed of large numbers of peptides, oligonucleotides or organic compounds and can be readily prepared by traditional automated synthesis methods, PCR, cloning or proprietary synthetic methods. Of particular interest are peptide and oligonucleotide combinatorial libraries. Still other libraries of interest include peptide, protein, peptidomimetic, multiparallel synthetic collection, recombinatorial, and polypeptide libraries. For a review of combinatorial chemistry and libraries created therefrom, see Myers, *Curr. Opin. Biotechnol.* 8:701-707 (1997). For reviews and examples of peptidomimetic libraries, see Al-Obeidi et al., *Mol. Biotechnol.*, 9(3):205-23 (1998); Hruby et al., *Curr Opin Chem Biol*, 1(1):114-19 (1997); Dorner et al., *Bioorg Med Chem*, 4(5):709-15 (1996) (alkylated dipeptides).

Identification of modulators through use of the various libraries described herein permits modification of the candidate "hit" (or "lead") to optimize the capacity of the "hit" to bind a polypeptide of the invention. The molecules identified in the binding assay are then tested for antagonist or agonist activity in *in vivo* tissue culture or animal models that are well known in the

art. In brief, the molecules are titrated into a plurality of cell cultures or animals and then tested for either cell/animal death or prolonged survival of the animal/cells.

The binding molecules thus identified may be complexed with toxins, *e.g.*, ricin or cholera, or with other compounds that are toxic to cells such as radioisotopes. The toxin-binding molecule complex is then targeted to a tumor or other cell by the specificity of the binding molecule for a polypeptide of the invention. Alternatively, the binding molecules may be complexed with imaging agents for targeting and imaging purposes.

4.10.14 ASSAY FOR RECEPTOR ACTIVITY

The invention also provides methods to detect specific binding of a polypeptide *e.g.* a ligand or a receptor. The art provides numerous assays particularly useful for identifying previously unknown binding partners for receptor polypeptides of the invention. For example, expression cloning using mammalian or bacterial cells, or dihybrid screening assays can be used to identify polynucleotides encoding binding partners. As another example, affinity chromatography with the appropriate immobilized polypeptide of the invention can be used to isolate polypeptides that recognize and bind polypeptides of the invention. There are a number of different libraries used for the identification of compounds, and in particular small molecules, that modulate (*i.e.*, increase or decrease) biological activity of a polypeptide of the invention. Ligands for receptor polypeptides of the invention can also be identified by adding exogenous ligands, or cocktails of ligands to two cells populations that are genetically identical except for the expression of the receptor of the invention: one cell population expresses the receptor of the invention whereas the other does not. The responses of the two cell populations to the addition of ligand(s) are then compared. Alternatively, an expression library can be co-expressed with the polypeptide of the invention in cells and assayed for an autocrine response to identify potential ligand(s). As still another example, BIAcore assays, gel overlay assays, or other methods known in the art can be used to identify binding partner polypeptides, including, (1) organic and inorganic chemical libraries, (2) natural product libraries, and (3) combinatorial libraries comprised of random peptides, oligonucleotides or organic molecules.

The role of downstream intracellular signaling molecules in the signaling cascade of the polypeptide of the invention can be determined. For example, a chimeric protein in which the cytoplasmic domain of the polypeptide of the invention is fused to the extracellular portion of a protein, whose ligand has been identified, is produced in a host cell. The cell is then incubated with the ligand specific for the extracellular portion of the chimeric protein, thereby activating the chimeric receptor. Known downstream proteins involved in intracellular signaling can then

be assayed for expected modifications *i.e.* phosphorylation. Other methods known to those in the art can also be used to identify signaling molecules involved in receptor activity.

4.10.15 ANTI-INFLAMMATORY ACTIVITY

5 Compositions of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cell-cell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting cell extravasation, or by stimulating or suppressing production
10 of other factors which more directly inhibit or promote an inflammatory response. Compositions with such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation intimation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or
15 chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of cytokines such as TNF or IL-1. Compositions of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material. Compositions of this invention may be utilized to prevent or treat conditions such as, but not limited to, sepsis, acute pancreatitis, endotoxin shock, cytokine induced shock, rheumatoid
20 arthritis, chronic inflammatory arthritis, pancreatic cell damage from diabetes mellitus type 1, graft versus host disease, inflammatory bowel disease, inflammation associated with pulmonary disease, other autoimmune disease or inflammatory disease, an antiproliferative agent such as for acute or chronic myelogenous leukemia or in the prevention of premature labor secondary to intrauterine infections.

4.10.16 LEUKEMIAS

Leukemias and related disorders may be treated or prevented by administration of a therapeutic that promotes or inhibits function of the polynucleotides and/or polypeptides of the invention. Such leukemias and related disorders include but are not limited to acute leukemia,
30 acute lymphocytic leukemia, acute myelocytic leukemia, myeloblastic, promyelocytic, myelomonocytic, monocytic, erythroleukemia, chronic leukemia, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia (for a review of such disorders, see Fishman et al., 1985, Medicine, 2d Ed., J.B. Lippincott Co., Philadelphia).

4.10.17 NERVOUS SYSTEM DISORDERS

Nervous system disorders, involving cell types which can be tested for efficacy of intervention with compounds that modulate the activity of the polynucleotides and/or polypeptides of the invention, and which can be treated upon thus observing an indication of therapeutic utility, include but are not limited to nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the invention include but are not limited to the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems:

- (i) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries;
- (ii) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia;
- (iii) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, syphilis;
- (iv) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis;
- (v) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration;
- (vi) neurological lesions associated with systemic diseases including but not limited to diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis;
- (vii) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and
- (viii) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including but not limited to multiple sclerosis, human

immunodeficiency virus-associated myelopathy, transverse myelopathy or various etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

Therapeutics which are useful according to the invention for treatment of a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, therapeutics which elicit any of the following effects may be useful according to the invention:

- (i) increased survival time of neurons in culture;
- (ii) increased sprouting of neurons in culture or *in vivo*;
- (iii) increased production of a neuron-associated molecule in culture or *in vivo*, *e.g.*, choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or
- (iv) decreased symptoms of neuron dysfunction *in vivo*.

Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may be measured by the method set forth in Arakawa et al. (1990, J. Neurosci. 10:3507-3515); increased sprouting of neurons may be detected by methods set forth in Pestronk et al. (1980, Exp. Neurol. 70:65-82) or Brown et al. (1981, Ann. Rev. Neurosci. 4:17-42); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, *etc.*, depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, *e.g.*, weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include but are not limited to disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including but not limited to progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

4.10.18 OTHER ACTIVITIES

A polypeptide of the invention may also exhibit one or more of the following additional activities or effects: inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye

color, skin, fat to lean ratio or other tissue pigmentation, or organ or body part size or shape (such as, for example, breast augmentation or diminution, change in bone form or shape); effecting biorhythms or circadian cycles or rhythms; effecting the fertility of male or female subjects; effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, co-factors or other nutritional factors or component(s); effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

4.10.19 IDENTIFICATION OF POLYMORPHISMS

The demonstration of polymorphisms makes possible the identification of such polymorphisms in human subjects and the pharmacogenetic use of this information for diagnosis and treatment. Such polymorphisms may be associated with, *e.g.*, differential predisposition or susceptibility to various disease states (such as disorders involving inflammation or immune response) or a differential response to drug administration, and this genetic information can be used to tailor preventive or therapeutic treatment appropriately. For example, the existence of a polymorphism associated with a predisposition to inflammation or autoimmune disease makes possible the diagnosis of this condition in humans by identifying the presence of the polymorphism.

Polymorphisms can be identified in a variety of ways known in the art which all generally involve obtaining a sample from a patient, analyzing DNA from the sample, optionally involving isolation or amplification of the DNA, and identifying the presence of the polymorphism in the DNA. For example, PCR may be used to amplify an appropriate fragment of genomic DNA which may then be sequenced. Alternatively, the DNA may be subjected to allele-specific oligonucleotide hybridization (in which appropriate oligonucleotides are hybridized to the DNA under conditions permitting detection of a single base mismatch) or to a single nucleotide extension assay (in which an oligonucleotide that hybridizes immediately adjacent to the position of the polymorphism is extended with one or more labeled nucleotides).

In addition, traditional restriction fragment length polymorphism analysis (using restriction enzymes that provide differential digestion of the genomic DNA depending on the presence or absence of the polymorphism) may be performed. Arrays with nucleotide sequences of the present invention can be used to detect polymorphisms. The array can comprise modified
5 nucleotide sequences of the present invention in order to detect the nucleotide sequences of the present invention. In the alternative, any one of the nucleotide sequences of the present invention can be placed on the array to detect changes from those sequences.

Alternatively a polymorphism resulting in a change in the amino acid sequence could also be detected by detecting a corresponding change in amino acid sequence of the protein, *e.g.*,
10 by an antibody specific to the variant sequence.

4.10.20 ARTHRITIS AND INFLAMMATION

The immunosuppressive effects of the compositions of the invention against rheumatoid arthritis are determined in an experimental animal model system. The experimental model
15 system is adjuvant induced arthritis in rats, and the protocol is described by J. Holoshitz, et al., 1983, Science, 219:56, or by B. Waksman et al., 1963, Int. Arch. Allergy Appl. Immunol., 23:129. Induction of the disease can be caused by a single injection, generally intradermally, of a suspension of killed Mycobacterium tuberculosis in complete Freund's adjuvant (CFA). The route of injection can vary, but rats may be injected at the base of the tail with an adjuvant
20 mixture. The polypeptide is administered in phosphate buffered solution (PBS) at a dose of about 1-5 mg/kg. The control consists of administering PBS only.

The procedure for testing the effects of the test compound would consist of intradermally injecting killed Mycobacterium tuberculosis in CFA followed by immediately administering the test compound and subsequent treatment every other day until day 24. At 14, 15, 18, 20, 22, and
25 24 days after injection of Mycobacterium CFA, an overall arthritis score may be obtained as described by J. Holoskitz above. An analysis of the data would reveal that the test compound would have a dramatic affect on the swelling of the joints as measured by a decrease of the arthritis score.

4.11 THERAPEUTIC METHODS

The compositions (including polypeptide fragments, analogs, variants and antibodies or other binding partners or modulators including antisense polynucleotides) of the invention have numerous applications in a variety of therapeutic methods. Examples of therapeutic applications include, but are not limited to, those exemplified herein.

4.11.1 EXAMPLE

One embodiment of the invention is the administration of an effective amount of the polypeptides or other composition of the invention to individuals affected by a disease or disorder that can be modulated by regulating the peptides of the invention. While the mode of administration is not particularly important, parenteral administration is preferred. An exemplary mode of administration is to deliver an intravenous bolus. The dosage of the polypeptides or other composition of the invention will normally be determined by the prescribing physician. It is to be expected that the dosage will vary according to the age, weight, condition and response of the individual patient. Typically, the amount of polypeptide administered per dose will be in the range of about 0.01 µg/kg to 100 mg/kg of body weight, with the preferred dose being about 0.1 µg/kg to 10 mg/kg of patient body weight. For parenteral administration, polypeptides of the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well known in the art and examples include water, saline, Ringer's solution, dextrose solution, and solutions consisting of small amounts of the human serum albumin. The vehicle may contain minor amounts of additives that maintain the isotonicity and stability of the polypeptide or other active ingredient. The preparation of such solutions is within the skill of the art.

4.12 PHARMACEUTICAL FORMULATIONS AND ROUTES OF ADMINISTRATION

A protein or other composition of the present invention (from whatever source derived, including without limitation from recombinant and non-recombinant sources and including antibodies and other binding partners of the polypeptides of the invention) may be administered to a patient in need, by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s) at doses to treat or ameliorate a variety of disorders. Such a composition may optionally contain (in addition to protein or other active ingredient and a carrier) diluents, fillers, salts, buffers, stabilizers, solubilizers, and other materials well known in the art. The term "pharmaceutically acceptable" means a non-toxic material that does not interfere with the effectiveness of the biological activity of the active ingredient(s). The characteristics of the carrier will depend on the route of administration. The pharmaceutical composition of the invention may also contain cytokines, lymphokines, or other hematopoietic factors such as M-CSF, GM-CSF, TNF, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IFN, TNF0, TNF1, TNF2, G-CSF, Meg-CSF, thrombopoietin, stem cell factor, and erythropoietin. In further compositions, proteins of the invention may be combined with other agents beneficial to the treatment of the disease or disorder in question. These agents

include various growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), insulin-like growth factor (IGF), as well as cytokines described herein.

The pharmaceutical composition may further contain other agents which either enhance the activity of the protein or other active ingredient or complement its activity or use in treatment. Such additional factors and/or agents may be included in the pharmaceutical composition to produce a synergistic effect with protein or other active ingredient of the invention, or to minimize side effects. Conversely, protein or other active ingredient of the present invention may be included in formulations of the particular clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent to minimize side effects of the clotting factor, cytokine, lymphokine, other hematopoietic factor, thrombolytic or anti-thrombotic factor, or anti-inflammatory agent (such as IL-1Ra, IL-1 Hy1, IL-1 Hy2, anti-TNF, corticosteroids, immunosuppressive agents). A protein of the present invention may be active in multimers (*e.g.*, heterodimers or homodimers) or complexes with itself or other proteins. As a result, pharmaceutical compositions of the invention may comprise a protein of the invention in such multimeric or complexed form.

As an alternative to being included in a pharmaceutical composition of the invention including a first protein, a second protein or a therapeutic agent may be concurrently administered with the first protein (*e.g.*, at the same time, or at differing times provided that therapeutic concentrations of the combination of agents is achieved at the treatment site). Techniques for formulation and administration of the compounds of the instant application may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition. A therapeutically effective dose further refers to that amount of the compound sufficient to result in amelioration of symptoms, *e.g.*, treatment, healing, prevention or amelioration of the relevant medical condition, or an increase in rate of treatment, healing, prevention or amelioration of such conditions. When applied to an individual active ingredient, administered alone, a therapeutically effective dose refers to that ingredient alone. When applied to a combination, a therapeutically effective dose refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

In practicing the method of treatment or use of the present invention, a therapeutically effective amount of protein or other active ingredient of the present invention is administered to a mammal having a condition to be treated. Protein or other active ingredient of the present invention may be administered in accordance with the method of the invention either alone or in combination with other therapies such as treatments employing cytokines, lymphokines or other

hematopoietic factors. When co-administered with one or more cytokines, lymphokines or other hematopoietic factors, protein or other active ingredient of the present invention may be administered either simultaneously with the cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors, or sequentially. If administered sequentially, the attending physician will decide on the appropriate sequence of administering protein or other active ingredient of the present invention in combination with cytokine(s), lymphokine(s), other hematopoietic factor(s), thrombolytic or anti-thrombotic factors.

4.12.1 ROUTES OF ADMINISTRATION

Suitable routes of administration may, for example, include oral, rectal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Administration of protein or other active ingredient of the present invention used in the pharmaceutical composition or to practice the method of the present invention can be carried out in a variety of conventional ways, such as oral ingestion, inhalation, topical application or cutaneous, subcutaneous, intraperitoneal, parenteral or intravenous injection. Intravenous administration to the patient is preferred.

Alternately, one may administer the compound in a local rather than systemic manner, for example, via injection of the compound directly into a arthritic joints or in fibrotic tissue, often in a depot or sustained release formulation. In order to prevent the scarring process frequently occurring as complication of glaucoma surgery, the compounds may be administered topically, for example, as eye drops. Furthermore, one may administer the drug in a targeted drug delivery system, for example, in a liposome coated with a specific antibody, targeting, for example, arthritic or fibrotic tissue. The liposomes will be targeted to and taken up selectively by the afflicted tissue.

The polypeptides of the invention are administered by any route that delivers an effective dosage to the desired site of action. The determination of a suitable route of administration and an effective dosage for a particular indication is within the level of skill in the art. Preferably for wound treatment, one administers the therapeutic compound directly to the site. Suitable dosage ranges for the polypeptides of the invention can be extrapolated from these dosages or from similar studies in appropriate animal models. Dosages can then be adjusted as necessary by the clinician to provide maximal therapeutic benefit.

4.12.2 COMPOSITIONS/FORMULATIONS

Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in a conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. These pharmaceutical compositions may be

5 manufactured in a manner that is itself known, *e.g.*, by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes. Proper formulation is dependent upon the route of administration chosen. When a therapeutically effective amount of protein or other active ingredient of the present invention is administered orally, protein or other active ingredient of the present
10 invention will be in the form of a tablet, capsule, powder, solution or elixir. When administered in tablet form, the pharmaceutical composition of the invention may additionally contain a solid carrier such as a gelatin or an adjuvant. The tablet, capsule, and powder contain from about 5 to 95% protein or other active ingredient of the present invention, and preferably from about 25 to 90% protein or other active ingredient of the present invention. When administered in liquid
15 form, a liquid carrier such as water, petroleum, oils of animal or plant origin such as peanut oil, mineral oil, soybean oil, or sesame oil, or synthetic oils may be added. The liquid form of the pharmaceutical composition may further contain physiological saline solution, dextrose or other saccharide solution, or glycols such as ethylene glycol, propylene glycol or polyethylene glycol. When administered in liquid form, the pharmaceutical composition contains from about 0.5 to
20 90% by weight of protein or other active ingredient of the present invention, and preferably from about 1 to 50% protein or other active ingredient of the present invention.

When a therapeutically effective amount of protein or other active ingredient of the present invention is administered by intravenous, cutaneous or subcutaneous injection, protein or other active ingredient of the present invention will be in the form of a pyrogen-free, parenterally
25 acceptable aqueous solution. The preparation of such parenterally acceptable protein or other active ingredient solutions, having due regard to pH, isotonicity, stability, and the like, is within the skill in the art. A preferred pharmaceutical composition for intravenous, cutaneous, or subcutaneous injection should contain, in addition to protein or other active ingredient of the present invention, an isotonic vehicle such as Sodium Chloride Injection, Ringer's Injection,
30 Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection, or other vehicle as known in the art. The pharmaceutical composition of the present invention may also contain stabilizers, preservatives, buffers, antioxidants, or other additives known to those of skill in the art. For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's
35 solution, or physiological saline buffer. For transmucosal administration, penetrants appropriate

to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained from a solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for such administration. For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the compounds for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.*, gelatin for use

in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch. The compounds may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides. In addition to the formulations described previously, the compounds may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

A pharmaceutical carrier for the hydrophobic compounds of the invention is a co-solvent system comprising benzyl alcohol, a nonpolar surfactant, a water-miscible organic polymer, and an aqueous phase. The co-solvent system may be the VPD co-solvent system. VPD is a solution of 3% w/v benzyl alcohol, 8% w/v of the nonpolar surfactant polysorbate 80, and 65% w/v polyethylene glycol 300, made up to volume in absolute ethanol. The VPD co-solvent system (VPD:5W) consists of VPD diluted 1:1 with a 5% dextrose in water solution. This co-solvent system dissolves hydrophobic compounds well, and itself produces low toxicity upon systemic administration. Naturally, the proportions of a co-solvent system may be varied considerably without destroying its solubility and toxicity characteristics. Furthermore, the identity of the co-solvent components may be varied: for example, other low-toxicity nonpolar surfactants may

be used instead of polysorbate 80; the fraction size of polyethylene glycol may be varied; other biocompatible polymers may replace polyethylene glycol, *e.g.* polyvinyl pyrrolidone; and other sugars or polysaccharides may substitute for dextrose. Alternatively, other delivery systems for hydrophobic pharmaceutical compounds may be employed. Liposomes and emulsions are well known examples of delivery vehicles or carriers for hydrophobic drugs. Certain organic solvents such as dimethylsulfoxide also may be employed, although usually at the cost of greater toxicity. Additionally, the compounds may be delivered using a sustained-release system, such as semipermeable matrices of solid hydrophobic polymers containing the therapeutic agent. Various types of sustained-release materials have been established and are well known by those skilled in the art. Sustained-release capsules may, depending on their chemical nature, release the compounds for a few weeks up to over 100 days. Depending on the chemical nature and the biological stability of the therapeutic reagent, additional strategies for protein or other active ingredient stabilization may be employed.

The pharmaceutical compositions also may comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include but are not limited to calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. Many of the active ingredients of the invention may be provided as salts with pharmaceutically compatible counter ions. Such pharmaceutically acceptable base addition salts are those salts which retain the biological effectiveness and properties of the free acids and which are obtained by reaction with inorganic or organic bases such as sodium hydroxide, magnesium hydroxide, ammonia, trialkylamine, dialkylamine, monoalkylamine, dibasic amino acids, sodium acetate, potassium benzoate, triethanol amine and the like.

The pharmaceutical composition of the invention may be in the form of a complex of the protein(s) or other active ingredient(s) of present invention along with protein or peptide antigens. The protein and/or peptide antigen will deliver a stimulatory signal to both B and T lymphocytes. B-lymphocytes will respond to antigen through their surface immunoglobulin receptor. T lymphocytes will respond to antigen through the T cell receptor (TCR) following presentation of the antigen by MHC proteins. MHC and structurally related proteins including those encoded by class I and class II MHC genes on host cells will serve to present the peptide antigen(s) to T lymphocytes. The antigen components could also be supplied as purified MHC-peptide complexes alone or with co-stimulatory molecules that can directly signal T cells. Alternatively antibodies able to bind surface immunoglobulin and other molecules on B cells as well as antibodies able to bind the TCR and other molecules on T cells can be combined with the pharmaceutical composition of the invention.

The pharmaceutical composition of the invention may be in the form of a liposome in which protein of the present invention is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids which exist in aggregated form as micelles, insoluble monolayers, liquid crystals, or lamellar layers in aqueous solution. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithins, phospholipids, saponin, bile acids, and the like. Preparation of such liposomal formulations is within the level of skill in the art, as disclosed, for example, in U.S. Patent Nos. 4,235,871; 4,501,728; 4,837,028; and 4,737,323, all of which are incorporated herein by reference.

The amount of protein or other active ingredient of the present invention in the pharmaceutical composition of the present invention will depend upon the nature and severity of the condition being treated, and on the nature of prior treatments which the patient has undergone. Ultimately, the attending physician will decide the amount of protein or other active ingredient of the present invention with which to treat each individual patient. Initially, the attending physician will administer low doses of protein or other active ingredient of the present invention and observe the patient's response. Larger doses of protein or other active ingredient of the present invention may be administered until the optimal therapeutic effect is obtained for the patient, and at that point the dosage is not increased further. It is contemplated that the various pharmaceutical compositions used to practice the method of the present invention should contain about 0.01 μ g to about 100 mg (preferably about 0.1 μ g to about 10 mg, more preferably about 0.1 μ g to about 1 mg) of protein or other active ingredient of the present invention per kg body weight. For compositions of the present invention which are useful for bone, cartilage, tendon or ligament regeneration, the therapeutic method includes administering the composition topically, systemically, or locally as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone, cartilage or tissue damage. Topical administration may be suitable for wound healing and tissue repair. Therapeutically useful agents other than a protein or other active ingredient of the invention which may also optionally be included in the composition as described above, may alternatively or additionally, be administered simultaneously or sequentially with the composition in the methods of the invention. Preferably for bone and/or cartilage formation, the composition would include a matrix capable of delivering the protein-containing or other active ingredient-containing composition to the site of bone and/or cartilage damage, providing a structure for the developing bone and cartilage and optimally

capable of being resorbed into the body. Such matrices may be formed of materials presently in use for other implanted medical applications.

The choice of matrix material is based on biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. The particular application of the compositions will define the appropriate formulation. Potential matrices for the compositions may be biodegradable and chemically defined calcium sulfate, tricalcium phosphate, hydroxyapatite, polylactic acid, polyglycolic acid and polyanhydrides. Other potential materials are biodegradable and biologically well-defined, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. Other potential matrices are nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics. Matrices may be comprised of combinations of any of the above-mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalcium phosphate. The bioceramics may be altered in composition, such as in calcium-aluminate-phosphate and processing to alter pore size, particle size, particle shape, and biodegradability. Presently preferred is a 50:50 (mole weight) copolymer of lactic acid and glycolic acid in the form of porous particles having diameters ranging from 150 to 800 microns. In some applications, it will be useful to utilize a sequestering agent, such as carboxymethyl cellulose or autologous blood clot, to prevent the protein compositions from disassociating from the matrix.

A preferred family of sequestering agents is cellulosic materials such as alkylcelluloses (including hydroxyalkylcelluloses), including methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropylcellulose, hydroxypropyl-methylcellulose, and carboxymethylcellulose, the most preferred being cationic salts of carboxymethylcellulose (CMC). Other preferred sequestering agents include hyaluronic acid, sodium alginate, poly(ethylene glycol), polyoxyethylene oxide, carboxyvinyl polymer and poly(vinyl alcohol). The amount of sequestering agent useful herein is 0.5-20 wt %, preferably 1-10 wt % based on total formulation weight, which represents the amount necessary to prevent desorption of the protein from the polymer matrix and to provide appropriate handling of the composition, yet not so much that the progenitor cells are prevented from infiltrating the matrix, thereby providing the protein the opportunity to assist the osteogenic activity of the progenitor cells. In further compositions, proteins or other active ingredients of the invention may be combined with other agents beneficial to the treatment of the bone and/or cartilage defect, wound, or tissue in question. These agents include various growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factors (TGF- α and TGF- β), and insulin-like growth factor (IGF).

The therapeutic compositions are also presently valuable for veterinary applications. Particularly domestic animals and thoroughbred horses, in addition to humans, are desired patients for such treatment with proteins or other active ingredients of the present invention. The dosage regimen of a protein-containing pharmaceutical composition to be used in tissue

5 regeneration will be determined by the attending physician considering various factors which modify the action of the proteins, *e.g.*, amount of tissue weight desired to be formed, the site of damage, the condition of the damaged tissue, the size of a wound, type of damaged tissue (*e.g.*, bone), the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution

10 and with inclusion of other proteins in the pharmaceutical composition. For example, the addition of other known growth factors, such as IGF I (insulin like growth factor I), to the final composition, may also effect the dosage. Progress can be monitored by periodic assessment of tissue/bone growth and/or repair, for example, X-rays, histomorphometric determinations and tetracycline labeling.

15 Polynucleotides of the present invention can also be used for gene therapy. Such polynucleotides can be introduced either in vivo or ex vivo into cells for expression in a mammalian subject. Polynucleotides of the invention may also be administered by other known methods for introduction of nucleic acid into a cell or organism (including, without limitation, in the form of viral vectors or naked DNA). Cells may also be cultured ex vivo in the presence of

20 proteins of the present invention in order to proliferate or to produce a desired effect on or activity in such cells. Treated cells can then be introduced in vivo for therapeutic purposes.

4.12.3 EFFECTIVE DOSAGE

Pharmaceutical compositions suitable for use in the present invention include

25 compositions wherein the active ingredients are contained in an effective amount to achieve its intended purpose. More specifically, a therapeutically effective amount means an amount effective to prevent development of or to alleviate the existing symptoms of the subject being treated. Determination of the effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein. For any compound used in

30 the method of the invention, the therapeutically effective dose can be estimated initially from appropriate in vitro assays. For example, a dose can be formulated in animal models to achieve a circulating concentration range that can be used to more accurately determine useful doses in humans. For example, a dose can be formulated in animal models to achieve a circulating concentration range that includes the IC₅₀ as determined in cell culture (*i.e.*, the concentration of

the test compound which achieves a half-maximal inhibition of the protein's biological activity). Such information can be used to more accurately determine useful doses in humans.

A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient. Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio between LD₅₀ and ED₅₀. Compounds which exhibit high therapeutic indices are preferred.

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. See, *e.g.*, Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1. Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety which are sufficient to maintain the desired effects, or minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from *in vitro* data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. However, HPLC assays or bioassays can be used to determine plasma concentrations.

Dosage intervals can also be determined using MEC value. Compounds should be administered using a regimen that maintains plasma levels above the MEC for 10-90% of the time, preferably between 30-90% and most preferably between 50-90%. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

An exemplary dosage regimen for polypeptides or other compositions of the invention will be in the range of about 0.01 µg/kg to 100 mg/kg of body weight daily, with the preferred dose being about 0.1 µg/kg to 25 mg/kg of patient body weight daily, varying in adults and children. Dosing may be once daily, or equivalent doses may be delivered at longer or shorter intervals.

The amount of composition administered will, of course, be dependent on the subject being treated, on the subject's age and weight, the severity of the affliction, the manner of administration and the judgment of the prescribing physician.

4.12.4 PACKAGING

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

4.13 ANTIBODIES

Also included in the invention are antibodies to proteins, or fragments of proteins of the invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, *i.e.*, molecules that contain an antigen-binding site that specifically binds (immunoreacts with) an antigen. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain, F_{ab} , F_{ab}' , and $F_{(ab)2}$ fragments, and an F_{ab} expression library. In general, an antibody molecule obtained from humans relates to any of the classes IgG, IgM, IgA, IgE and IgD, which differ from one another by the nature of the heavy chain present in the molecule. Certain classes have subclasses as well, such as IgG₁, IgG₂, and others. Furthermore, in humans, the light chain may be a kappa chain or a lambda chain. Reference herein to antibodies includes a reference to all such classes, subclasses and types of human antibody species.

An isolated related protein of the invention may be intended to serve as an antigen, or a portion or fragment thereof, and additionally can be used as an immunogen to generate antibodies that immunospecifically bind the antigen, using standard techniques for polyclonal and monoclonal antibody preparation. The full-length protein can be used or, alternatively, the invention provides antigenic peptide fragments of the antigen for use as immunogens. An antigenic peptide fragment comprises at least 6 amino acid residues of the amino acid sequence of the full length protein, (for example the amino acid sequence shown in SEQ ID NO: 30369), and encompasses an epitope thereof such that an antibody raised against the peptide forms a specific immune complex with the full length protein or with any fragment that contains the epitope. Preferably, the antigenic peptide comprises at least 10 amino acid residues, or at least 15 amino acid residues, or at least 20 amino acid residues, or at least 30 amino acid residues. Preferred epitopes encompassed by the antigenic peptide are regions of the protein that are located on its surface; commonly these are hydrophilic regions.

In certain embodiments of the invention, at least one epitope encompassed by the antigenic peptide is a region on the surface of the protein of the invention that is located on the

surface of the protein, *e.g.*, a hydrophilic region. A hydrophobicity analysis of the human related protein sequence will indicate which regions of a related protein are particularly hydrophilic and, therefore, are likely to encode surface residues useful for targeting antibody production. As a means for targeting antibody production, hydropathy plots showing regions of hydrophilicity and hydrophobicity may be generated by any method well known in the art, including, for example, the Kyte Doolittle or the Hopp Woods methods, either with or without Fourier transformation. See, *e.g.*, Hopp and Woods, 1981, *Proc. Nat. Acad. Sci. USA* 78: 3824-3828; Kyte and Doolittle 1982, *J. Mol. Biol.* 157: 105-142, each of which is incorporated herein by reference in its entirety. Antibodies that are specific for one or more domains within an antigenic protein, or derivatives, fragments, analogs or homologs thereof, are also provided herein.

A protein of the invention, or a derivative, fragment, analog, homolog or ortholog thereof, may be utilized as an immunogen in the generation of antibodies that immunospecifically bind these protein components.

Various procedures known within the art may be used for the production of polyclonal or monoclonal antibodies directed against a protein of the invention, or against derivatives, fragments, analogs homologs or orthologs thereof (see, for example, *Antibodies: A Laboratory Manual*, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, incorporated herein by reference). Some of these antibodies are discussed below.

5.13.1 Polyclonal Antibodies

For the production of polyclonal antibodies, various suitable host animals (*e.g.*, rabbit, goat, mouse or other mammal) may be immunized by one or more injections with the native protein, a synthetic variant thereof, or a derivative of the foregoing. An appropriate immunogenic preparation can contain, for example, the naturally occurring immunogenic protein, a chemically synthesized polypeptide representing the immunogenic protein, or a recombinantly expressed immunogenic protein. Furthermore, the protein may be conjugated to a second protein known to be immunogenic in the mammal being immunized. Examples of such immunogenic proteins include but are not limited to keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, and soybean trypsin inhibitor. The preparation can further include an adjuvant. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, etc.), adjuvants usable in humans such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory agents. Additional examples of

adjuvants which can be employed include MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate).

The polyclonal antibody molecules directed against the immunogenic protein can be isolated from the mammal (*e.g.*, from the blood) and further purified by well known techniques, such as affinity chromatography using protein A or protein G, which provide primarily the IgG fraction of immune serum. Subsequently, or alternatively, the specific antigen which is the target of the immunoglobulin sought, or an epitope thereof, may be immobilized on a column to purify the immune specific antibody by immunoaffinity chromatography. Purification of immunoglobulins is discussed, for example, by D. Wilkinson (The Scientist, published by The Scientist, Inc., Philadelphia PA, Vol. 14, No. 8 (April 17, 2000), pp. 25-28).

5.13.2 Monoclonal Antibodies

The term "monoclonal antibody" (MAb) or "monoclonal antibody composition", as used herein, refers to a population of antibody molecules that contain only one molecular species of antibody molecule consisting of a unique light chain gene product and a unique heavy chain gene product. In particular, the complementarity determining regions (CDRs) of the monoclonal antibody are identical in all the molecules of the population. MAbs thus contain an antigen-binding site capable of immunoreacting with a particular epitope of the antigen characterized by a unique binding affinity for it.

Monoclonal antibodies can be prepared using hybridoma methods, such as those described by Kohler and Milstein, Nature, 256:495 (1975). In a hybridoma method, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes can be immunized in vitro.

The immunizing agent will typically include the protein antigen, a fragment thereof or a fusion protein thereof. Generally, either peripheral blood lymphocytes are used if cells of human origin are desired, or spleen cells or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice, Academic Press, (1986) pp. 59-103). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells can be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the

culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, California and the American Type Culture Collection, Manassas, Virginia. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, J. Immunol., 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, Marcel Dekker, Inc., New York, (1987) pp. 51-63).

The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA). Such techniques and assays are known in the art. The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem., 107:220 (1980). Preferably, antibodies having a high degree of specificity and a high binding affinity for the target antigen are isolated.

After the desired hybridoma cells are identified, the clones can be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium and RPMI-1640 medium. Alternatively, the hybridoma cells can be grown in vivo as ascites in a mammal.

The monoclonal antibodies secreted by the subclones can be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

The monoclonal antibodies can also be made by recombinant DNA methods, such as those described in U.S. Patent No. 4,816,567. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells such as simian COS cells, Chinese hamster ovary (CHO) cells, or

myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, Nature **368**, 812-13 (1994)) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

5.13.2 Humanized Antibodies

The antibodies directed against the protein antigens of the invention can further comprise humanized antibodies or human antibodies. These antibodies are suitable for administration to humans without engendering an immune response by the human against the administered immunoglobulin. Humanized forms of antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) that are principally comprised of the sequence of a human immunoglobulin, and contain minimal sequence derived from a non-human immunoglobulin. Humanization can be performed following the method of Winter and co-workers (Jones et al., Nature, **321**:522-525 (1986); Riechmann et al., Nature, **332**:323-327 (1988); Verhoeyen et al., Science, **239**:1534-1536 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. (See also U.S. Patent No. 5,225,539.) In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies can also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., 1986; Riechmann et al., 1988; and Presta, Curr. Op. Struct. Biol., **2**:593-596 (1992)).

5.13.3 Human Antibodies

Fully human antibodies relate to antibody molecules in which essentially the entire sequences of both the light chain and the heavy chain, including the CDRs, arise from human genes. Such antibodies are termed "human antibodies", or "fully human antibodies" herein. Human monoclonal antibodies can be prepared by the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized in the practice of the present invention and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., 1985 In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96).

In addition, human antibodies can also be produced using additional techniques, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991)). Similarly, human antibodies can be made by introducing human immunoglobulin loci into transgenic animals, *e.g.*, mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly, and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; 5,661,016, and in Marks et al. (Bio/Technology 10, 779-783 (1992)); Lonberg et al. (Nature 368 856-859 (1994)); Morrison (Nature 368, 812-13 (1994)); Fishwild et al. (Nature Biotechnology 14, 845-51 (1996)); Neuberger (Nature Biotechnology 14, 826 (1996)); and Lonberg and Huszar (Intern. Rev. Immunol. 13 65-93 (1995)).

Human antibodies may additionally be produced using transgenic nonhuman animals which are modified so as to produce fully human antibodies rather than the animal's endogenous antibodies in response to challenge by an antigen. (See PCT publication WO94/02602). The endogenous genes encoding the heavy and light immunoglobulin chains in the nonhuman host have been incapacitated, and active loci encoding human heavy and light chain immunoglobulins are inserted into the host's genome. The human genes are incorporated, for example, using yeast artificial chromosomes containing the requisite human DNA segments. An animal which provides all the desired modifications is then obtained as progeny by crossbreeding intermediate transgenic animals containing fewer than the full complement of the modifications. The preferred embodiment of such a nonhuman animal is a mouse, and is termed the XenomouseTM as disclosed in PCT publications WO 96/33735 and WO 96/34096. This animal produces B cells which secrete fully human immunoglobulins. The antibodies can be obtained directly from

the animal after immunization with an immunogen of interest, as, for example, a preparation of a polyclonal antibody, or alternatively from immortalized B cells derived from the animal, such as hybridomas producing monoclonal antibodies. Additionally, the genes encoding the immunoglobulins with human variable regions can be recovered and expressed to obtain the antibodies directly, or can be further modified to obtain analogs of antibodies such as, for example, single chain Fv molecules.

An example of a method of producing a nonhuman host, exemplified as a mouse, lacking expression of an endogenous immunoglobulin heavy chain is disclosed in U.S. Patent No. 5,939,598. It can be obtained by a method including deleting the J segment genes from at least one endogenous heavy chain locus in an embryonic stem cell to prevent rearrangement of the locus and to prevent formation of a transcript of a rearranged immunoglobulin heavy chain locus, the deletion being effected by a targeting vector containing a gene encoding a selectable marker; and producing from the embryonic stem cell a transgenic mouse whose somatic and germ cells contain the gene encoding the selectable marker.

A method for producing an antibody of interest, such as a human antibody, is disclosed in U.S. Patent No. 5,916,771. It includes introducing an expression vector that contains a nucleotide sequence encoding a heavy chain into one mammalian host cell in culture, introducing an expression vector containing a nucleotide sequence encoding a light chain into another mammalian host cell, and fusing the two cells to form a hybrid cell. The hybrid cell expresses an antibody containing the heavy chain and the light chain.

In a further improvement on this procedure, a method for identifying a clinically relevant epitope on an immunogen, and a correlative method for selecting an antibody that binds immunospecifically to the relevant epitope with high affinity, are disclosed in PCT publication WO 99/53049.

5.13.4 F_{ab} Fragments and Single Chain Antibodies

According to the invention, techniques can be adapted for the production of single-chain antibodies specific to an antigenic protein of the invention (see *e.g.*, U.S. Patent No. 4,946,778). In addition, methods can be adapted for the construction of F_{ab} expression libraries (see *e.g.*, Huse, et al., 1989 Science 246: 1275-1281) to allow rapid and effective identification of monoclonal F_{ab} fragments with the desired specificity for a protein or derivatives, fragments, analogs or homologs thereof. Antibody fragments that contain the idiotypes to a protein antigen may be produced by techniques known in the art including, but not limited to: (i) an F_{(ab')₂} fragment produced by pepsin digestion of an antibody molecule; (ii) an F_{ab} fragment generated

by reducing the disulfide bridges of an $F_{(ab)2}$ fragment; (iii) an F_{ab} fragment generated by the treatment of the antibody molecule with papain and a reducing agent and (iv) F_v fragments.

5.13.5 Bispecific Antibodies

5 Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have binding specificities for at least two different antigens. In the present case, one of the binding specificities is for an antigenic protein of the invention. The second binding target is any other antigen, and advantageously is a cell-surface protein or receptor or receptor subunit.

Methods for making bispecific antibodies are known in the art. Traditionally, the
10 recombinant production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy-chain/light-chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of ten different antibody molecules, of which only one has the correct
15 bispecific structure. The purification of the correct molecule is usually accomplished by affinity chromatography steps. Similar procedures are disclosed in WO 93/08829, published 13 May 1993, and in Traunecker *et al.*, 1991 *EMBO J.*, 10:3655-3659.

Antibody variable domains with the desired binding specificities (antibody-antigen combining sites) can be fused to immunoglobulin constant domain sequences. The fusion
20 preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, CH2, and CH3 regions. It is preferred to have the first heavy-chain constant region (CH1) containing the site necessary for light-chain binding present in at least one of the fusions. DNAs encoding the immunoglobulin heavy-chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable
25 host organism. For further details of generating bispecific antibodies see, for example, Suresh *et al.*, Methods in Enzymology, 121:210 (1986).

According to another approach described in WO 96/27011, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the
30 CH3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.* tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.* alanine or threonine). This provides a mechanism for
35 increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Bispecific antibodies can be prepared as full length antibodies or antibody fragments (*e.g.* F(ab')₂ bispecific antibodies). Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., Science 229:81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Additionally, Fab' fragments can be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., J. Exp. Med. 175:217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., J. Immunol. 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., Proc. Natl. Acad. Sci. USA 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the V_H and V_L domains of one fragment are forced to pair with the complementary V_L and V_H domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See, Gruber et al., J. Immunol. 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

Exemplary bispecific antibodies can bind to two different epitopes, at least one of which originates in the protein antigen of the invention. Alternatively, an anti-antigenic arm of an immunoglobulin molecule can be combined with an arm which binds to a triggering molecule on a leukocyte such as a T-cell receptor molecule (*e.g.* CD2, CD3, CD28, or B7), or Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the cell expressing the particular antigen. Bispecific antibodies can also be used to direct cytotoxic agents to cells which express a particular antigen. These antibodies possess an antigen-binding arm and an arm which binds a cytotoxic agent or a radionuclide chelator, such as EOTUBE, DPTA, DOTA, or TETA. Another bispecific antibody of interest binds the protein antigen described herein and further binds tissue factor (TF).

5.13.6 Heteroconjugate Antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/200373; EP 03089). It is contemplated that the antibodies can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980.

5.13.7 Effector Function Engineering

It can be desirable to modify the antibody of the invention with respect to effector function, so as to enhance, *e.g.*, the effectiveness of the antibody in treating cancer. For example, cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated can have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al., *J. Exp Med.*, 176: 1191-1195 (1992) and Shopes, *J. Immunol.*, 148: 2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity can also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research*, 53: 2560-2565 (1993). Alternatively, an antibody can

be engineered that has dual Fc regions and can thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al., *Anti-Cancer Drug Design*, 3: 219-230 (1989).

5.13.8 Immunoconjugates

5 The invention also pertains to immunoconjugates comprising an antibody conjugated to a cytotoxic agent such as a chemotherapeutic agent, toxin (*e.g.*, an enzymatically active toxin of bacterial, fungal, plant, or animal origin, or fragments thereof), or a radioactive isotope (*i.e.*, a radioconjugate).

Chemotherapeutic agents useful in the generation of such immunoconjugates have been described above. Enzymatically active toxins and fragments thereof that can be used include diphtheria A chain, nonbinding active fragments of diphtheria toxin, exotoxin A chain (from *Pseudomonas aeruginosa*), ricin A chain, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, *Phytolacca americana* proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, *saponaria officinalis* inhibitor, gelonin, 10 mitogellin, restrictocin, phenomycin, enomycin, and the tricothecenes. A variety of radionuclides are available for the production of radioconjugated antibodies. Examples include ^{212}Bi , ^{131}I , ^{131}In , ^{90}Y , and ^{186}Re .

Conjugates of the antibody and cytotoxic agent are made using a variety of bifunctional protein-coupling agents such as N-succinimidyl-3-(2-pyridyldithiol) propionate (SPDP), 20 iminothiolane (IT), bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutaraldehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis-diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4-dinitrobenzene). For example, a 25 ricin immunotoxin can be prepared as described in Vitetta et al., *Science*, 238: 1098 (1987). Carbon-14-labeled 1-isothiocyanatobenzyl-3-methyldiethylene triaminepentaacetic acid (MX-DTPA) is an exemplary chelating agent for conjugation of radionucleotide to the antibody. See WO94/11026.

In another embodiment, the antibody can be conjugated to a "receptor" (such 30 streptavidin) for utilization in tumor pretargeting wherein the antibody-receptor conjugate is administered to the patient, followed by removal of unbound conjugate from the circulation using a clearing agent and then administration of a "ligand" (*e.g.*, avidin) that is in turn conjugated to a cytotoxic agent.

35 4.14 COMPUTER READABLE SEQUENCES

In one application of this embodiment, a nucleotide sequence of the present invention can be recorded on computer readable media. As used herein, "computer readable media" refers to any medium which can be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc storage medium, and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM and ROM; and hybrids of these categories such as magnetic/optical storage media. A skilled artisan can readily appreciate how any of the presently known computer readable mediums can be used to create a manufacture comprising computer readable medium having recorded thereon a nucleotide sequence of the present invention. As used herein, "recorded" refers to a process for storing information on computer readable medium. A skilled artisan can readily adopt any of the presently known methods for recording information on computer readable medium to generate manufactures comprising the nucleotide sequence information of the present invention.

A variety of data storage structures are available to a skilled artisan for creating a computer readable medium having recorded thereon a nucleotide sequence of the present invention. The choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats can be used to store the nucleotide sequence information of the present invention on computer readable medium. The sequence information can be represented in a word processing text file, formatted in commercially-available software such as WordPerfect and Microsoft Word, or represented in the form of an ASCII file, stored in a database application, such as DB2, Sybase, Oracle, or the like. A skilled artisan can readily adapt any number of data processor structuring formats (*e.g.* text file or database) in order to obtain computer readable medium having recorded thereon the nucleotide sequence information of the present invention.

By providing any of the nucleotide sequences SEQ ID NO: 1-30368 or a representative fragment thereof; or a nucleotide sequence at least 95% identical to any of the nucleotide sequences of SEQ ID NO: 1-30368 in computer readable form, a skilled artisan can routinely access the sequence information for a variety of purposes. Computer software is publicly available which allows a skilled artisan to access sequence information provided in a computer readable medium. The examples which follow demonstrate how software which implements the BLAST (Altschul et al., J. Mol. Biol. 215:403-410 (1990)) and BLAZE (Brutlag et al., Comp. Chem. 17:203-207 (1993)) search algorithms on a Sybase system is used to identify open reading frames (ORFs) within a nucleic acid sequence. Such ORFs may be protein encoding fragments and may be useful in producing commercially important proteins such as enzymes used in fermentation reactions and in the production of commercially useful metabolites.

As used herein, "a computer-based system" refers to the hardware means, software means, and data storage means used to analyze the nucleotide sequence information of the present invention. The minimum hardware means of the computer-based systems of the present invention comprises a central processing unit (CPU), input means, output means, and data storage means. A skilled artisan can readily appreciate that any one of the currently available computer-based systems are suitable for use in the present invention. As stated above, the computer-based systems of the present invention comprise a data storage means having stored therein a nucleotide sequence of the present invention and the necessary hardware means and software means for supporting and implementing a search means. As used herein, "data storage means" refers to memory which can store nucleotide sequence information of the present invention, or a memory access means which can access manufactures having recorded thereon the nucleotide sequence information of the present invention.

As used herein, "search means" refers to one or more programs which are implemented on the computer-based system to compare a target sequence or target structural motif with the sequence information stored within the data storage means. Search means are used to identify fragments or regions of a known sequence which match a particular target sequence or target motif. A variety of known algorithms are disclosed publicly and a variety of commercially available software for conducting search means are and can be used in the computer-based systems of the present invention. Examples of such software includes, but is not limited to, Smith-Waterman, MacPattern (EMBL), BLASTN and BLASTA (NPOLYPEPTIDEIA). A skilled artisan can readily recognize that any one of the available algorithms or implementing software packages for conducting homology searches can be adapted for use in the present computer-based systems. As used herein, a "target sequence" can be any nucleic acid or amino acid sequence of six or more nucleotides or two or more amino acids. A skilled artisan can readily recognize that the longer a target sequence is, the less likely a target sequence will be present as a random occurrence in the database. The most preferred sequence length of a target sequence is from about 10 to 300 amino acids, more preferably from about 30 to 100 nucleotide residues. However, it is well recognized that searches for commercially important fragments, such as sequence fragments involved in gene expression and protein processing, may be of shorter length.

As used herein, "a target structural motif," or "target motif," refers to any rationally selected sequence or combination of sequences in which the sequence(s) are chosen based on a three-dimensional configuration which is formed upon the folding of the target motif. There are a variety of target motifs known in the art. Protein target motifs include, but are not limited to, enzyme active sites and signal sequences. Nucleic acid target motifs include, but are not limited

to, promoter sequences, hairpin structures and inducible expression elements (protein binding sequences).

4.15 TRIPLE HELIX FORMATION

5 In addition, the fragments of the present invention, as broadly described, can be used to control gene expression through triple helix formation or antisense DNA or RNA, both of which methods are based on the binding of a polynucleotide sequence to DNA or RNA.

Polynucleotides suitable for use in these methods are preferably 20 to 40 bases in length and are designed to be complementary to a region of the gene involved in transcription (triple helix - see
10 Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 15241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Olmno, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA
transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA
15 molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide.

4.16 DIAGNOSTIC ASSAYS AND KITS

20 The present invention further provides methods to identify the presence or expression of one of the ORFs of the present invention, or homolog thereof, in a test sample, using a nucleic acid probe or antibodies of the present invention, optionally conjugated or otherwise associated with a suitable label.

In general, methods for detecting a polynucleotide of the invention can comprise
25 contacting a sample with a compound that binds to and forms a complex with the polynucleotide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a polynucleotide of the invention is detected in the sample. Such methods can also comprise contacting a sample under stringent hybridization conditions with nucleic acid primers that anneal to a polynucleotide of the invention under such conditions, and amplifying annealed
30 polynucleotides, so that if a polynucleotide is amplified, a polynucleotide of the invention is detected in the sample.

In general, methods for detecting a polypeptide of the invention can comprise contacting a sample with a compound that binds to and forms a complex with the polypeptide for a period sufficient to form the complex, and detecting the complex, so that if a complex is detected, a
35 polypeptide of the invention is detected in the sample.

In detail, such methods comprise incubating a test sample with one or more of the antibodies or one or more of the nucleic acid probes of the present invention and assaying for binding of the nucleic acid probes or antibodies to components within the test sample.

Conditions for incubating a nucleic acid probe or antibody with a test sample vary.

5 Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the nucleic acid probe or antibody used in the assay. One skilled in the art will recognize that any one of the commonly available hybridization, amplification or immunological assay formats can readily be adapted to employ the nucleic acid probes or antibodies of the present invention. Examples of such assays can be found in Chard,
10 T., *An Introduction to Radioimmunoassay and Related Techniques*, Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock, G.R. et al., *Techniques in Immunocytochemistry*, Academic Press, Orlando, FL Vol. 1 (1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, P., *Practice and Theory of immunoassays: Laboratory Techniques in Biochemistry and Molecular Biology*, Elsevier Science Publishers, Amsterdam, The Netherlands (1985). The test samples of the
15 present invention include cells, protein or membrane extracts of cells, or biological fluids such as sputum, blood, serum, plasma, or urine. The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily be adapted in order to obtain a
20 sample which is compatible with the system utilized.

In another embodiment of the present invention, kits are provided which contain the necessary reagents to carry out the assays of the present invention. Specifically, the invention provides a compartment kit to receive, in close confinement, one or more containers which comprises: (a) a first container comprising one of the probes or antibodies of the present
25 invention; and (b) one or more other containers comprising one or more of the following: wash reagents, reagents capable of detecting presence of a bound probe or antibody.

In detail, a compartment kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allows one to efficiently transfer reagents from one compartment to
30 another compartment such that the samples and reagents are not cross-contaminated, and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the antibodies used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, etc.), and containers which
35 contain the reagents used to detect the bound antibody or probe. Types of detection reagents

include labeled nucleic acid probes, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. One skilled in the art will readily recognize that the disclosed probes and antibodies of the present invention can be readily incorporated into one of the established kit formats which are well known in the art.

4.17 MEDICAL IMAGING

The novel polypeptides and binding partners of the invention are useful in medical imaging of sites expressing the molecules of the invention (*e.g.*, where the polypeptide of the invention is involved in the immune response, for imaging sites of inflammation or infection). See, *e.g.*, Kunkel et al., U.S. Pat. NO. 5,413,778. Such methods involve chemical attachment of a labeling or imaging agent, administration of the labeled polypeptide to a subject in a pharmaceutically acceptable carrier, and imaging the labeled polypeptide *in vivo* at the target site.

4.18 SCREENING ASSAYS

Using the isolated proteins and polynucleotides of the invention, the present invention further provides methods of obtaining and identifying agents which bind to a polypeptide encoded by an ORF corresponding to any of the nucleotide sequences set forth in SEQ ID NO: 1-30368, or bind to a specific domain of the polypeptide encoded by the nucleic acid. In detail, said method comprises the steps of:

- (a) contacting an agent with an isolated protein encoded by an ORF of the present invention, or nucleic acid of the invention; and
- (b) determining whether the agent binds to said protein or said nucleic acid.

In general, therefore, such methods for identifying compounds that bind to a polynucleotide of the invention can comprise contacting a compound with a polynucleotide of the invention for a time sufficient to form a polynucleotide/compound complex, and detecting the complex, so that if a polynucleotide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Likewise, in general, therefore, such methods for identifying compounds that bind to a polypeptide of the invention can comprise contacting a compound with a polypeptide of the invention for a time sufficient to form a polypeptide/compound complex, and detecting the complex, so that if a polypeptide/compound complex is detected, a compound that binds to a polynucleotide of the invention is identified.

Methods for identifying compounds that bind to a polypeptide of the invention can also comprise contacting a compound with a polypeptide of the invention in a cell for a time sufficient to form a polypeptide/compound complex, wherein the complex drives expression of a receptor gene sequence in the cell, and detecting the complex by detecting reporter gene sequence expression, so that if a polypeptide/compound complex is detected, a compound that binds a polypeptide of the invention is identified.

Compounds identified via such methods can include compounds which modulate the activity of a polypeptide of the invention (that is, increase or decrease its activity, relative to activity observed in the absence of the compound). Alternatively, compounds identified via such methods can include compounds which modulate the expression of a polynucleotide of the invention (that is, increase or decrease expression relative to expression levels observed in the absence of the compound). Compounds, such as compounds identified via the methods of the invention, can be tested using standard assays well known to those of skill in the art for their ability to modulate activity/expression.

The agents screened in the above assay can be, but are not limited to, peptides, carbohydrates, vitamin derivatives, or other pharmaceutical agents. The agents can be selected and screened at random or rationally selected or designed using protein modeling techniques.

For random screening, agents such as peptides, carbohydrates, pharmaceutical agents and the like are selected at random and are assayed for their ability to bind to the protein encoded by the ORF of the present invention. Alternatively, agents may be rationally selected or designed. As used herein, an agent is said to be "rationally selected or designed" when the agent is chosen based on the configuration of the particular protein. For example, one skilled in the art can readily adapt currently available procedures to generate peptides, pharmaceutical agents and the like, capable of binding to a specific peptide sequence, in order to generate rationally designed antipeptide peptides, for example see Hurby et al., Application of Synthetic Peptides: Antisense Peptides," In Synthetic Peptides, A User's Guide, W.H. Freeman, NY (1992), pp. 289-307, and Kaspczak et al., Biochemistry 28:9230-8 (1989), or pharmaceutical agents, or the like.

In addition to the foregoing, one class of agents of the present invention, as broadly described, can be used to control gene expression through binding to one of the ORFs or EMFs of the present invention. As described above, such agents can be randomly screened or rationally designed/selected. Targeting the ORF or EMF allows a skilled artisan to design sequence specific or element specific agents, modulating the expression of either a single ORF or multiple ORFs which rely on the same EMF for expression control. One class of DNA binding agents are agents which contain base residues which hybridize or form a triple helix formation by binding to DNA or RNA. Such agents can be based on the classic phosphodiester,

ribonucleic acid backbone, or can be a variety of sulfhydryl or polymeric derivatives which have base attachment capacity.

Agents suitable for use in these methods preferably contain 20 to 40 bases and are designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). Triple helix-formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. Both techniques have been demonstrated to be effective in model systems. Information contained in the sequences of the present invention is necessary for the design of an antisense or triple helix oligonucleotide and other DNA binding agents.

Agents that bind to a protein encoded by one of the ORFs of the present invention can be used as a diagnostic agent. Agents which bind to a protein encoded by one of the ORFs of the present invention can be formulated using known techniques to generate a pharmaceutical composition.

4.19 USE OF NUCLEIC ACIDS AS PROBES

Another aspect of the subject invention is to provide for polypeptide-specific nucleic acid hybridization probes capable of hybridizing with naturally occurring nucleotide sequences. The hybridization probes of the subject invention may be derived from any of the nucleotide sequences SEQ ID NO: 1-30368. Because the corresponding gene is only expressed in a limited number of tissues, a hybridization probe derived from any of the nucleotide sequences SEQ ID NO: 1-30368 can be used as an indicator of the presence of RNA of cell type of such a tissue in a sample.

Any suitable hybridization technique can be employed, such as, for example, *in situ* hybridization. PCR as described in US Patents Nos. 4,683,195 and 4,965,188 provides additional uses for oligonucleotides based upon the nucleotide sequences. Such probes used in PCR may be of recombinant origin, may be chemically synthesized, or a mixture of both. The probe will comprise a discrete nucleotide sequence for the detection of identical sequences or a degenerate pool of possible sequences for identification of closely related genomic sequences.

Other means for producing specific hybridization probes for nucleic acids include the cloning of nucleic acid sequences into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA

polymerase and the appropriate radioactively labeled nucleotides. The nucleotide sequences may be used to construct hybridization probes for mapping their respective genomic sequences. The nucleotide sequence provided herein may be mapped to a chromosome or specific regions of a chromosome using well known genetic and/or chromosomal mapping techniques. These techniques include in situ hybridization, linkage analysis against known chromosomal markers, hybridization screening with libraries or flow-sorted chromosomal preparations specific to known chromosomes, and the like. The technique of fluorescent in situ hybridization of chromosome spreads has been described, among other places, in Verma et al (1988) Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York NY.

Fluorescent *in situ* hybridization of chromosomal preparations and other physical chromosome mapping techniques may be correlated with additional genetic map data. Examples of genetic map data can be found in the 1994 Genome Issue of Science (265:1981f). Correlation between the location of a nucleic acid on a physical chromosomal map and a specific disease (or predisposition to a specific disease) may help delimit the region of DNA associated with that genetic disease. The nucleotide sequences of the subject invention may be used to detect differences in gene sequences between normal, carrier or affected individuals.

4.20 PREPARATION OF SUPPORT BOUND OLIGONUCLEOTIDES

Oligonucleotides, *i.e.*, small nucleic acid segments, may be readily prepared by, for example, directly synthesizing the oligonucleotide by chemical means, as is commonly practiced using an automated oligonucleotide synthesizer.

Support bound oligonucleotides may be prepared by any of the methods known to those of skill in the art using any suitable support such as glass, polystyrene or Teflon. One strategy is to precisely spot oligonucleotides synthesized by standard synthesizers. Immobilization can be achieved using passive adsorption (Inouye & Hondo, (1990) J. Clin. Microbiol. 28(6) 1469-72); using UV light (Nagata *et al.*, 1985; Dahlen *et al.*, 1987; Morrissey & Collins, (1989) Mol. Cell Probes 3(2) 189-207) or by covalent binding of base modified DNA (Keller *et al.*, 1988; 1989); all references being specifically incorporated herein.

Another strategy that may be employed is the use of the strong biotin-streptavidin interaction as a linker. For example, Broude *et al.* (1994) Proc. Natl. Acad. Sci. USA 91(8) 3072-6, describe the use of biotinylated probes, although these are duplex probes, that are immobilized on streptavidin-coated magnetic beads. Streptavidin-coated beads may be purchased from Dynal, Oslo. Of course, this same linking chemistry is applicable to coating any surface with streptavidin. Biotinylated probes may be purchased from various sources, such as, *e.g.*, Operon Technologies (Alameda, CA).

Nunc Laboratories (Naperville, IL) is also selling suitable material that could be used. Nunc Laboratories have developed a method by which DNA can be covalently bound to the microwell surface termed CovaLink NH. CovaLink NH is a polystyrene surface grafted with secondary amino groups (>NH) that serve as bridge-heads for further covalent coupling. CovaLink Modules may be purchased from Nunc Laboratories. DNA molecules may be bound to CovaLink exclusively at the 5'-end by a phosphoramidate bond, allowing immobilization of more than 1 pmol of DNA (Rasmussen *et al.*, (1991) *Anal. Biochem.* 198(1) 138-42).

The use of CovaLink NH strips for covalent binding of DNA molecules at the 5'-end has been described (Rasmussen *et al.*, (1991). In this technology, a phosphoramidate bond is employed (Chu *et al.*, (1983) *Nucleic Acids Res.* 11(8) 6513-29). This is beneficial as immobilization using only a single covalent bond is preferred. The phosphoramidate bond joins the DNA to the CovaLink NH secondary amino groups that are positioned at the end of spacer arms covalently grafted onto the polystyrene surface through a 2 nm long spacer arm. To link an oligonucleotide to CovaLink NH via an phosphoramidate bond, the oligonucleotide terminus must have a 5'-end phosphate group. It is, perhaps, even possible for biotin to be covalently bound to CovaLink and then streptavidin used to bind the probes.

More specifically, the linkage method includes dissolving DNA in water (7.5 ng/ul) and denaturing for 10 min. at 95°C and cooling on ice for 10 min. Ice-cold 0.1 M 1-methylimidazole, pH 7.0 (1-MeIm₇), is then added to a final concentration of 10 mM 1-MeIm₇. A ss DNA solution is then dispensed into CovaLink NH strips (75 ul/well) standing on ice.

Carbodiimide 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), dissolved in 10 mM 1-MeIm₇, is made fresh and 25 ul added per well. The strips are incubated for 5 hours at 50°C. After incubation the strips are washed using, *e.g.*, Nunc-Immuno Wash; first the wells are washed 3 times, then they are soaked with washing solution for 5 min., and finally they are washed 3 times (where in the washing solution is 0.4 N NaOH, 0.25% SDS heated to 50°C).

It is contemplated that a further suitable method for use with the present invention is that described in PCT Patent Application WO 90/03382 (Southern & Maskos), incorporated herein by reference. This method of preparing an oligonucleotide bound to a support involves attaching a nucleoside 3'-reagent through the phosphate group by a covalent phosphodiester link to aliphatic hydroxyl groups carried by the support. The oligonucleotide is then synthesized on the supported nucleoside and protecting groups removed from the synthetic oligonucleotide chain under standard conditions that do not cleave the oligonucleotide from the support. Suitable reagents include nucleoside phosphoramidite and nucleoside hydrogen phosphate.

An on-chip strategy for the preparation of DNA probe for the preparation of DNA probe arrays may be employed. For example, addressable laser-activated photodeprotection may be

employed in the chemical synthesis of oligonucleotides directly on a glass surface, as described by Fodor *et al.* (1991) *Science* 251(4995) 767-73, incorporated herein by reference. Probes may also be immobilized on nylon supports as described by Van Ness *et al.* (1991) *Nucleic Acids Res.* 19(12) 3345-50; or linked to Teflon using the method of Duncan & Cavalier (1988) *Anal. Biochem.* 169(1) 104-8; all references being specifically incorporated herein.

To link an oligonucleotide to a nylon support, as described by Van Ness *et al.* (1991), requires activation of the nylon surface via alkylation and selective activation of the 5'-amine of oligonucleotides with cyanuric chloride.

One particular way to prepare support bound oligonucleotides is to utilize the light-generated synthesis described by Pease *et al.*, (1994) *PNAS USA* 91(11) 5022-6, incorporated herein by reference). These authors used current photolithographic techniques to generate arrays of immobilized oligonucleotide probes (DNA chips). These methods, in which light is used to direct the synthesis of oligonucleotide probes in high-density, miniaturized arrays, utilize photolabile 5'-protected *N*-acyl-deoxynucleoside phosphoramidites, surface linker chemistry and versatile combinatorial synthesis strategies. A matrix of 256 spatially defined oligonucleotide probes may be generated in this manner.

4.21 PREPARATION OF NUCLEIC ACID FRAGMENTS

The nucleic acids may be obtained from any appropriate source, such as cDNAs, genomic DNA, chromosomal DNA, microdissected chromosome bands, cosmid or YAC inserts, and RNA, including mRNA without any amplification steps. For example, Sambrook *et al.* (1989) describes three protocols for the isolation of high molecular weight DNA from mammalian cells (p. 9.14-9.23).

DNA fragments may be prepared as clones in M13, plasmid or lambda vectors and/or prepared directly from genomic DNA or cDNA by PCR or other amplification methods. Samples may be prepared or dispensed in multiwell plates. About 100-1000 ng of DNA samples may be prepared in 2-500 µl of final volume.

The nucleic acids would then be fragmented by any of the methods known to those of skill in the art including, for example, using restriction enzymes as described at 9.24-9.28 of Sambrook *et al.* (1989), shearing by ultrasound and NaOH treatment.

Low pressure shearing is also appropriate, as described by Schriefer *et al.* (1990) *Nucleic Acids Res.* 18(24) 7455-6, incorporated herein by reference). In this method, DNA samples are passed through a small French pressure cell at a variety of low to intermediate pressures. A lever device allows controlled application of low to intermediate pressures to the cell. The results of

these studies indicate that low-pressure shearing is a useful alternative to sonic and enzymatic DNA fragmentation methods.

One particularly suitable way for fragmenting DNA is contemplated to be that using the two base recognition endonuclease, *Cvi*II, described by Fitzgerald *et al.* (1992) Nucleic Acids Res.

20(14) 3753-62. These authors described an approach for the rapid fragmentation and fractionation of DNA into particular sizes that they contemplated to be suitable for shotgun cloning and sequencing.

The restriction endonuclease *Cvi*II normally cleaves the recognition sequence PuGCPy between the G and C to leave blunt ends. Atypical reaction conditions, which alter the specificity of this enzyme (*Cvi*II**), yield a quasi-random distribution of DNA fragments from the small molecule pUC19 (2688 base pairs). Fitzgerald *et al.* (1992) quantitatively evaluated the randomness of this fragmentation strategy, using a *Cvi*II** digest of pUC19 that was size fractionated by a rapid gel filtration method and directly ligated, without end repair, to a lac Z minus M13 cloning vector. Sequence analysis of 76 clones showed that *Cvi*II** restricts pyGCPy and PuGCPu, in addition to PuGCPy sites, and that new sequence data is accumulated at a rate consistent with random fragmentation.

As reported in the literature, advantages of this approach compared to sonication and agarose gel fractionation include: smaller amounts of DNA are required (0.2-0.5 ug instead of 2-5 ug); and fewer steps are involved (no preligation, end repair, chemical extraction, or agarose gel electrophoresis and elution are needed).

Irrespective of the manner in which the nucleic acid fragments are obtained or prepared, it is important to denature the DNA to give single stranded pieces available for hybridization. This is achieved by incubating the DNA solution for 2-5 minutes at 80-90°C. The solution is then cooled quickly to 2°C to prevent renaturation of the DNA fragments before they are contacted with the chip. Phosphate groups must also be removed from genomic DNA by methods known in the art.

4.22 PREPARATION OF DNA ARRAYS

Arrays may be prepared by spotting DNA samples on a support such as a nylon membrane. Spotting may be performed by using arrays of metal pins (the positions of which correspond to an array of wells in a microtiter plate) to repeated by transfer of about 20 nl of a DNA solution to a nylon membrane. By offset printing, a density of dots higher than the density of the wells is achieved. One to 25 dots may be accommodated in 1 mm², depending on the type of label used. By avoiding spotting in some preselected number of rows and columns, separate subsets (subarrays) may be formed. Samples in one subarray may be the same genomic segment of DNA (or the same gene) from different individuals, or may be different, overlapped genomic clones. Each of the

subarrays may represent replica spotting of the same samples. In one example, a selected gene segment may be amplified from 64 patients. For each patient, the amplified gene segment may be in one 96-well plate (all 96 wells containing the same sample). A plate for each of the 64 patients is prepared. By using a 96-pin device, all samples may be spotted on one 8 x 12 cm membrane.

5 Subarrays may contain 64 samples, one from each patient. Where the 96 subarrays are identical, the dot span may be 1 mm² and there may be a 1 mm space between subarrays.

Another approach is to use membranes or plates (available from NUNC, Naperville, Illinois) which may be partitioned by physical spacers *e.g.* a plastic grid molded over the membrane, the grid being similar to the sort of membrane applied to the bottom of multiwell plates, or hydrophobic
10 strips. A fixed physical spacer is not preferred for imaging by exposure to flat phosphor-storage screens or x-ray films.

The present invention is illustrated in the following examples. Upon consideration of the present disclosure, one of skill in the art will appreciate that many other embodiments and variations may be made in the scope of the present invention. Accordingly, it is intended that the broader
15 aspects of the present invention not be limited to the disclosure of the following examples. The present invention is not to be limited in scope by the exemplified embodiments which are intended as illustrations of single aspects of the invention, and compositions and methods which are functionally equivalent are within the scope of the invention. Indeed, numerous modifications and variations in the practice of the invention are expected to occur to those skilled in the art upon
20 consideration of the present preferred embodiments. Consequently, the only limitations which should be placed upon the scope of the invention are those which appear in the appended claims.

All references cited within the body of the instant specification are hereby incorporated by reference in their entirety.

5.0 EXAMPLES

25 5.1 EXAMPLE 1

Novel Nucleic Acid Sequences Obtained From Various Libraries

A plurality of novel nucleic acids were obtained from cDNA libraries prepared from various human tissues and in some cases isolated from a genomic library derived from human chromosome using standard PCR, SBH sequence signature analysis and Sanger sequencing techniques. The
30 inserts of the library were amplified with PCR using primers specific for the vector sequences which flank the inserts. Clones from cDNA libraries were spotted on nylon membrane filters and screened with oligonucleotide probes (*e.g.*, 7-mers) to obtain signature sequences. The clones were clustered into groups of similar or identical sequences. Representative clones were selected for sequencing.

In some cases, the 5' sequence of the amplified inserts was then deduced using a typical Sanger sequencing protocol. PCR products were purified and subjected to fluorescent dye terminator cycle sequencing. Single pass gel sequencing was done using a 377 Applied Biosystems (ABI) sequencer to obtain the novel nucleic acid sequences. In some cases RACE (Rapid Amplification of cDNA Ends) was performed to further extend the sequence in the 5' direction.

5.2 EXAMPLE 2

Novel Contigs

The novel contigs of the invention were assembled from sequences that were obtained from a cDNA library by methods described in Example 1 above, and in some cases sequences obtained from one or more public databases. The sequences for the resulting nucleic acid contigs are designated as SEQ ID NO: 1-30368 and are provided in the attached Sequence Listing. The contigs were assembled using an EST sequence as a seed. Then a recursive algorithm was used to extend the seed EST into an extended assemblage, by pulling additional sequences from different databases (*i.e.*, Hyseq's database containing EST sequences, dbEST version 115, gb pri 115, and UniGene version 103, and exons from public domain genomic sequences predicted by GenScan) that belong to this assemblage. The algorithm terminated when there was no additional sequences from the above databases that would extend the assemblage. Further, the inclusion of component sequences into the assemblage was based on a BLASTN hit to the extending assemblage with BLAST score greater than 300 and percent identity greater than 95%.

The novel predicted polypeptides (including proteins) encoded by the novel polynucleotides (SEQ ID NO: 1-30368) of the present invention are incorporated in the attached Sequence Listing. A subset the predicted polypeptide sequences contain an unknown amino acid, a stop codon, a possible nucleotide deletion or a possible nucleotide insertion. These sequences have been shown in their entirety with the special characters in Table 2. Table 2 also shows the corresponding start and stop nucleotide locations to each of SEQ ID NO: 1-30368. Table 2 also indicates the method by which the polypeptide was predicted. Method A refers to a polypeptide obtained by using a software program called FASTY (available from <http://fasta.bioch.virginia.edu>) which selects a polypeptide based on a comparison of the translated novel polynucleotide to known polynucleotides (W.R. Pearson, Methods in Enzymology, 183:63-98 (1990), herein incorporated by reference). Method B refers to a polypeptide obtained by using a software program called GenScan for human/vertebrate sequences (available from Stanford University, Office of Technology Licensing) that predicts the polypeptide based on a probabilistic model of gene structure/compositional properties (C. Burge and S. Karlin, J. Mol. Biol., 268:78-94 (1997), incorporated herein by

reference). Method C refers to a polypeptide obtained by using a Hyseq proprietary software program that translates the novel polynucleotide and its complementary strand into six possible amino acid sequences (forward and reverse frames) and chooses the polypeptide with the longest open reading frame.

5 The nearest neighbor results for SEQ ID NO: 1-30368 were obtained by a BLASTP version 2.0a1 19MP-WashU search against Genpept release 121 and Geneseq release 200103 (Derwent), using BLAST algorithm. The nearest neighbor result showed the closest homologue for SEQ ID NO: 1-30368. The nearest neighbor results for SEQ ID NO: 1-30368 are incorporated in the attached Sequence Listing.

10 Using eMatrix software package (Stanford University, Stanford, CA) (Wu et al., J. Comp. Biol., Vol. 6 pp. 219-235 (1999) herein incorporated by reference), all the sequences were examined to determine whether they had identifiable signature regions. The attached Sequence Listing provides the results obtained by eMatrix analysis for each polypeptide as follows: the signature region found in the indicated polypeptide sequences, the description of the signature,
15 the eMatrix p-value(s) and the position(s) of the signature within the polypeptide sequence.

 Using the pFam software program (Sonnhammer et al., Nucleic Acids Res., Vol. 26(1) pp. 320-322 (1998) herein incorporated by reference) all the polypeptide sequences were examined for domains with homology to certain peptide domains. The attached Sequence Listing provides the results obtained by PFAM analysis for each peptide, namely: the name of
20 the domain found, the description, the p-value and the pFam score for the identified domain within the sequence.

 Tables 1 and 2 follow. Table 1 shows the various tissue sources of SEQ ID NO: 1-30368. Table 2 shows the start and stop nucleotides for the translated amino acid sequence for which each assemblage encodes. Table 2 also provides a correlation between the amino acid sequences set forth
25 in the Sequence Listing, the nucleotide sequences set forth in the Sequence Listing and the SEQ ID NO: in USSN 09/540,217

Table I

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
adult brain	GIBCO	AB3001	39-41 192 197-200 315-316 540-542 576-580 608-622 635 1004 1185-1187 1273-1279 1431 1474 1721-1722 2036 2136-2137 2457 2471-2474 2513 2599-2603 2988- 2989 3105-3106 3212 3276-3277 3306-3308 3352 3365 3374-3376 3433 3448-3450 3555-3558 3693 3949-3953 4067-4072 4160-4162 4558-4560 4581-4582 4612-4614 4837-4840 5483-5484 5603-5606 5700 5802 5980-5984 6135-6136 6403-6404 6452-6453 7209-7212 7447-7449 7452-7460 7536-7541 7554-7555 7622-7623 7630-7636 7660-7665 7701-7703 7771 7778-7783 7798-7801 7921- 7923 7994 8010-8012 8025-8026 8145-8151 8227-8229 8415 8497-8499 8936-8938 8986-8991 9002-9004 9013- 9017 9337-9338 9366-9368 9375-9376 9391-9392 9395- 9396 9431-9436 9443 9475-9476 9517-9518 9522-9525 9586-9589 9603-9604 9851-9852 9854-9855 9874-9895 9905-9908 9947-9952 9969-9980 9986-9992 10025- 10026 10033-10037 10167-10172 10277 10480-10482 10488-10489 10498-10503 10520-10522 10537-10538 10592-10594 10628-10630 11226-11227 11339-11344 11406-11407 11431-11432 11731-11734 12150-12151 12239 12241-12244 12555-12559 12615-12618 12785- 12787 12978-12981 12984-12985 12997-12999 13567- 13568 13592-13595 13606-13608 13873-13875 13999- 14004 14360-14369 14650-14651 14684-14685 15013- 15018 15096 15174-15181 15209-15210 15250-15251 15257 15323-15324 15548-15552 15568-15572 15576- 15577 15588-15589 15699-15700 15881-15883 16438- 16439 16473-16478 16496-16497 16609-16611 16686- 16693 16700-16701 16727-16729 16836-16842 16934- 16937 16949-16953 17455-17456 17857-17861 17958- 17963 18029-18030 18136-18138 18423-18425 18516- 18518 18535-18537 18624-18626 18668-18672 18719- 18722 18750-18756 18790-18793 18802-18804 18836- 18838 18899-18903 18919-18921 18943-18945 18947- 18950 18964-18969 18989-18990 19013-19017 19045- 19048 19057-19065 19142-19147 19154-19155 19224 19316-19317 19345-19349 19355-19360 19362 19370 19385-19389 19415-19417 19422-19431 19442-19444 19503 19560-19562 19566 19604-19607 19693 19709- 19710 19727-19732 19736-19742 19772 19804-19808 19921-19929 19933-19938 19943-19946 19969-19981 20015-20017 20029-20043 20087-20094 20099-20102 20111-20112 20122-20127 20161-20164 20167-20171 20180-20181 20189-20194 20198-20199 20215-20218 20281-20282 20289 20321-20324 20349-20354 20361 20393-20400 20415-20417 20437-20440 20524-20535 20542-20545 20554-20558 20607-20612 20614-20615 20646-20652 20698-20707 20718-20725 20727-20732 20789-20791 20806-20812 20844-20849 20888-20889 20926 20938-20942 20999-21004 21027-21031 21062- 21066 21072-21075 21137-21140 21145-21148 21153-

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
			21154 21272-21274 21277-21283 21410-21414 21434- 21439 21485-21491 21495-21500 21647-21655 21729- 21733 21929-21935 21958-21961 21973-21974 21978 22000-22006 22026-22029 22040-22041 22087-22088 22101-22107 22141-22143 22160 22250-22252 22284- 22289 22309 22314-22317 22336-22342 22347-22348 22358-22359 22372 22405-22408 22495 22534-22539 22634-22643 22653-22654 22661-22662 22665-22667 22671-22674 22700-22701 22794-22796 22805-22809 22887-22891 22899-22900 22948-22950 22952-22953 22982-22986 22991-22994 23059-23060 23071 23141 23249 23251 23329-23337 23412-23414 23489-23490 23492-23493 23508-23509 23543-23544 23704 23834- 23835 23890-23892 23959 24014-24018 25289-25290 25319-25321 25374-25375 25966-25968 26205-26206 26258-26259 26303 26316-26321 26327 26337 26373- 26374 26596-26601 26788-26789 26843 26850-26852 26897 27067-27070 27100-27102 27150-27151 27247- 27251 27304-27305 27439-27440 27493-27495 27636- 27639 27750-27754 27814-27818 27861-27864 27890- 27892 27989-27990 28099-28100 28311-28313 28424 28426-28428 29278-29283 29409-29416 29444 29718- 29721 30141-30142
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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Genomic DNA from BAC 63I18	Research Genetics (CITB BAC Library)	BAC001	3338-3339 4122-4123 4175-4176 4180-4190 4911-4914 5021-5023 5030-5035 5045-5046 5154-5179 7065-7066 7370-7374 7426-7442 8802-8806 8836-8838 8855-8859 8877-8891 9087-9099 9152-9192 9194-9195 9213-9215 9217-9225 9239-9257 9259 9269-9276 9291-9293 9301- 9304 9312-9313 10443-10444 10684-10689 10691-10703 10723-10724 10726-10727 10835-10839 10842-10867 10879-10880 12218-12220 13130-13132 13145 13176- 13178 13324-13325 13422-13423 13677-13680 13683- 13685 13693-13694 13708-13718 13823 13854-13860 14663-14665 15718-15725 15815-15817 15827 15910- 15916 15934-15950 15978-15980 15991-16000 16024- 16027 16232 17637-17641 17673 17688-17696 17738- 17739 17748-17751 17763-17781 17783-17786 17842-

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Genomic DNA from BAC 39316	Research Genetics (CITB BAC Library)	BAC002	4007 4037-4042 4177-4190 4193-4199 4905-4907 5030 5036-5046 5326-5330 7036-7037 7043-7055 7089-7091 7094-7099 7175-7177 7374 7392-7395 7407-7414 7421-7442 7444-7446 8466-8476 8710-8715 8802-8806 8839-8841 8855-8859 8866-8868 9150-9151 9182-9193 9201-9208 9213-9229 9231-9234 9258-9259 9277-9286 9291-9296 9300 9312-9313 10324-10325 10330-10331 10349-10350 10443-10444 10704-10718 10723-10724 10726-10750 10755-10769 10773-10777 10835-10839 10842-10867 10879-10880 11890-11891 13176 13184-13188 13324-13325 13701 13706-13736 13751 13815-13818 13823 13825-13831 13854-13860 14962 14981-14983 15110-15114 15718-15725 15838-15841 15847-15854 15910-15911 15939-15943 15951-15960 15964-15982 15991-16000 16016-16018 16024-16027 17275-17277 17409-17430 17637-17641 17688-17696 17699-17700 17748-17751 17771-17776 17783-17786 17805-17806 17813-17817 17819-17824 17828-17829 17837-17841 17843-17847 17871-17873 17876-17879 17888-17890

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Genomic DNA from BAC 393I6	Research Genetics (CITB BAC Library)	BAC003	1578-1585 4052 4205 4899-4903 4908-4910 5031-5032 5042 5334 6796-6800 7061-7064 7067-7087 7091 7094- 7099 7374 7392-7395 7426-7442 7467-7476 8839-8841 8859 8862 8899-8902 9145-9149 9152-9181 9259 9269- 9304 10443-10444 10759-10764 10819-10868 11076- 11077 13130-13132 13183 13751 13815-13832 15112 15120-15123 15718-15725 15847-15854 15944-15945 15966-15968 15991-16018 16021 17609 17653-17667 17683-17684 17734-17736 17771-17776 17783-17786 17791-17799 17805-17806 17827 17837-17841 17843- 17847 17862-17868 18010-18012 18075-18080 18152- 18154 18175-18183 18211-18214 18216-18218 18230- 18239 18255-18258 18275-18370 21304-21325 21501- 21515 21728 21788-21799 22498-22522 22525-22530 22582-22598 22603-22606 22610-22617 22702 23029-

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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bone marrow	Clontech	BMD007	373-374 4174 4362-4365 8320-8322 9531 15908-15909 16044-16046 16652 17160-17167 18771-18772 19749-19751 19814-19815 20698-20707 22310-22311 23070 25370-25372 26266 27702-27706
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Mixture of 16 tissues – mRNAs*	Various Vendors*	CTL016	373-374 847-848 4581-4582 7465-7466 7745-7746 9267-9268 13638-13641 14344-14345 15277-15278 15356-15357 18825-18828 19049-19053 19306-19307 19370 19706-19708 20263-20264 20345-20348 20425-20426

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Mixture of 16 tissues – mRNAs*	Various Vendors*	CTL021	1667-1668 4206-4208 4562-4568 5335-5336 5370 5919-5921 7256-7257 8434-8448 9984-9985 10588-10591 10870-10872 10965 12149 12714-12715 15126-15129 15530-15531 15543-15544 15576-15577 16652 16894-16896 18371-18372 18516-18518 19049-19053 19211-19212 19283-19294 19401-19402 19422-19431 19706-19708 19736-19742 19749-19751 19813 20289 21155-21156 21208-21210 22101-22107 22309 22358-22359 22373 22632-22633 22690 23074-23076 27544-27545 27593-27594 27989-27990 29332-29337 30135-30140
adult cervix	BioChain	CVX001	30 48-49 51-52 144-145 156 180-181 201 221 255-256 301 329-330 332 395 419-420 425-427 515-517 524-525 530-531 543-547 551-552 694-695 716-720 738-740 832-836 901-904 934-935 1001-1003 1047 1185-1187 1259-1265 1273-1279 1302-1303 1424-1429 1431 1453-1454 1456-1460 1474 1483 1499-1502 1563-1564 1596 1667-1668 1705-1714 1800 1866-1872 1887-1889 1899-1900 2015-2018 2036 2062 2220-2227 2275-2276 2307 2375 2404-2406 2431 2471-2474 2516 2540-2541 2599-2603 2626-2642 2644-2651 2653-2658 2661-2673 2692-2694 2710 2802 2921-2930 2951-2955 2988-2989 3001 3090 3205-3207 3245-3246 3268-3269 3290-3303 3421-3422 3455-3456 3466 3507-3510 3512-3520 3526-3529 3531-3534 3555-3558 3661-3670 3705-3706 3763-3765 3817 3949-3953 4122-4123 4160-4162 4221-4238 4469-4470 4599-4601 4663-4665 4692-4694 4762-4763 4785-4789 4795-4809 4819-4823 4957 5084-5085 5092-5094 5109-5115 5124-5126 5133 5335-5336 5364 5483-5484 5581-5583 5654 5766-5767 5932-5936 5973-5975 5980-5984 5997-6000 6011-6021 6284 6371-6376 6403-6404 6484 6486-6489 6624-6626 6779 6877-6881 6932-6938 6980 7192-7193 7209-7212 7216-7219 7279-7282 7294-7295 7350-7352 7534-7550 7576-7577 7601-7603 7609 7640-7642 7671-7673 7678-7689 7693-7694 7701-7703 7728 7742-7743 7747-7751 7771 7778-7783 7787-7788 7794-7801 7890 7924-7925 7976 7988 8020 8025-8026 8070 8084-8085 8115-8121 8132-8141 8145-8156 8184-8186 8258-8262 8270-8276 8313-8314 8342 8347-8360 8368-8370 8383-8389 8415 8422-8425 8430-8433 8466-8476 8504-8506 8543-8550 8688 8751-8753 8758-8759 8869 8917-8933 8982-8983 8986-8991 9021 9029-9035 9037 9072-9074 9196-9200 9305 9321-9323 9337-9338 9391-9392 9427-9428 9444 9456-9458 9487-9488 9506-9509 9522-9525 9531-9534 9617-9626 9684-9687 9709 9729-9731 9763-9767 9794-9795 9815-9820 9826 9828 9854-9856 9899-9901 9916-9920 9923-9924 9958-9961 9969-9980 9989-9992 9997-10009 10017-10019 10038-10040 10175-10196 10273-10274 10277 10306 10451-10453 10484-10489 10498-10503 10508-10515 10523-10532 10539-10542 10588-10594 10873-10875 10877-10878 10886-10889 10901-10902 10979 11013-11017 11028-11031 11147-11156 11217-11219 11252-11273 11300-11303 11317 11345-11350 11423-11426 11431-11432

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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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endothelial cells	Stratagene	EDT001	19-20 84 124-125 156 158-159 164 170-171 180-181 192 197-200 227-230 251-252 255-256 260 301 307-308 315-316 319-323 329-330 358-359 373-374 395 414-418 425-427 461-465 505 515-519 524-525 534-535 553-554 557-559 576-578 619-620 635 656 706-707 712 716-722 832-836 845-848 880-885 891 913 919-922 981-985 1004-1005 1044-1045 1047 1074-1087 1092-1098 1101-1103 1123-1127 1158-1161 1203-1204 1235-1237 1240-1241 1259-1266 1268-1289 1307 1310-1311 1334 1414-1417 1431 1453-1454 1467-1469 1483 1499 1569-1573 1618 1620 1667-1668 1678-1680 1705-1714 1721-1722 1778-1779 1792-1794 1910-1913 1925-1927 1967 1971-1974 1977 1986-1987 2015-2018 2022 2072 2202-2203 2272-2276 2306 2404-2406 2409 2432-2435 2471-2474 2570-2574 2597-2603 2625 2724-2726 2886-2887 2903-2904 2923-2924 2926 2934-2935 2939-2943 2975-2977 2983-2986 3001 3006-3008 3078-3079 3090 3107-3108 3115 3226-3228 3241 3255-3259 3266 3276-3277 3304-3309 3336-3337 3365 3374-3377 3421-3422 3433 3448-3450 3455-3456 3464-3465 3477-3478 3504-3507 3518-3520 3525 3555-3558 3606 3624 3686 3693 3781-3784 3789-3794 3834-3835 3949-3953 4067-4072 4080-4082 4084-4085 4122-4123 4160-4162 4213-4214 4244-4247 4343-4346 4450 4469-4482 4486-4488 4526-4527 4542 4555-4556 4562-4573 4581-4582 4600-4601 4612-4614 4651 4785-4789 4857-4861 4966-4968 4971-4974 5089-5090 5092-5094 5192 5335-5336 5384-5386 5572-5573 5580-5583 5594-5602 5658 5700 5714-5716 5802-5812 5820-5823 5880 5885-5897 5907-5909 5939-5941 5980-5984 5986-5988 6140-6143 6166-6168 6186-6189 6301 6342-6343 6403-6405 6551-6567 6585-6592 6627-6628 6657-6661 6669-6670 6791-6794 6832-6850 6852 6932-6938 7056-7057 7209-7212 7216-7219 7285-7295 7374 7415-7420 7447-7449 7536-7541 7557-7559 7580-7597 7601-7605 7609 7615-7617 7620-7621 7630-7636 7643-7647 7654-7655 7657-7665 7671-7673 7681-7687 7693-7696 7701-7703 7729 7742-7743 7745-7751 7784-7786 7794-7797 7821 7890 7906-7912 7921-7925 7946-7948 7972-7975 7978-7983 7990-7992 7994 8002 8010-8012 8017-8019 8043-8044 8070 8090-8093 8117-8121 8152-8159 8163-8173 8182-8183 8187-8200 8211-8213 8216-8220 8227-8229 8234 8247-8248 8253-8257 8342 8347-8360 8363-8364 8368-8370 8415 8449-8452 8497-8499 8504-8505 8507-8509 8512-8513 8539-8542 8554-8555 8584-8587 8607-8609 8688-8690 8754-8757 8760-8761 8777-8779 8936-8938 8986-8991 9002-9004 9013-9020 9038-9045 9072-9074 9084-9085 9196-9200 9260-9263 9306-9311 9316 9321-9323 9331-9333 9337-9338 9382 9391-

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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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fetal brain	Clontech	FBR001	202-203 847-848 1097-1098 1259-1262 1721-1722 2404-2406 2951-2955 5276-5278 5802 7902-7903 8377-8379 9196-9200 9443 9828 9969-9980 10273-10274 10326-10328 10876 10976-10978 11024-11025 11476-11478 11731-11734 11803-11804 12127-12128 12150-12151 13107-13117 13581-13583 14604 17366-17368 17455-17456 18627-18628 18964-18969 19018 19211-19212 19362 19387-19389 19401-19402 20328-20330 20345-20348 20554-20557 21256-21266 21377-21397 21434-21439 21978 22141-22143 22200-22203 22637-22643 22899-22900 23222 23709 23893-23902 25416-25417 26307-26309 26329 26831 27113 27245-27246 27386-27389 27976-27982 28186 30141-30142
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fetal brain	Clontech	FBR006	3-4 168 192 197-200 240-250 324-325 329-330 362 373- 374 464-465 532-535 553-554 576-578 613-614 713-715 847-848 912 927 934-935 949-950 1044-1045 1071 1097- 1098 1203-1204 1235-1237 1273-1279 1304 1411-1413 1488-1489 1569-1570 1591-1592 1678-1687 1705-1714 1923-1924 1977 2023-2029 2145 2228 2231-2253 2259- 2264 2356 2375 2396-2400 2404-2406 2431 2437-2439 2475 2525-2528 2599-2603 2656-2658 2663-2665 2707- 2716 2720-2732 2734-2745 2770-2772 2808-2811 2871- 2873 2889-2891 2931-2935 2951-2955 3001 3039 3080- 3081 3105-3106 3205-3207 3213 3261-3263 3377 3477- 3478 3507 3512-3514 3555-3558 3596 3671-3673 3683 3687-3691 3693 3708 3711-3712 3729-3730 3781-3784 3809 3939-3941 3949-3953 4055-4061 4065 4091-4100 4122-4126 4137-4139 4209-4210 4542 4562-4568 4574- 4576 4667 4673 4683-4684 4720-4725 4765-4773 4795- 4809 4845-4851 4854-4856 4870-4871 4948 4964-4965 4970 5136-5137 5139 5246 5251-5252 5291-5294 5392 5532-5533 5557-5560 5567-5568 5594-5602 5744-5747 6011-6021 6137 6155-6161 6209-6211 6217-6222 6378- 6388 6393-6395 6406-6410 6452-6453 6488-6490 6513- 6515 6542-6543 6669-6670 6674-6675 6775-6778 7194- 7197 7220-7227 7236-7237 7264-7266 7350-7352 7364- 7365 7426-7442 7452-7460 7482-7517 7557-7559 7580- 7597 7604-7605 7630-7636 7657-7659 7695-7696 7745- 7746 7778-7783 7787-7788 7898-7900 7946 7957 7986- 7987 7993 8013-8016 8079 8137-8141 8152-8156 8162- 8173 8187-8200 8204-8205 8211-8213 8230-8233 8247- 8249 8263-8265 8301-8310 8313-8314 8320-8322 8335- 8336 8347-8348 8351-8360 8371-8374 8383-8389 8420- 8421 8426-8428 8457-8458 8461-8465 8497-8499 8506 8512-8513 8588-8597 8607-8609 8688 8733-8735 8758- 8759 8762-8766 8919-8933 8936-8945 8974-8977 8982- 8983 8998-9004 9029-9030 9043-9045 9068 9306-9311 9380-9381 9510-9518 9529-9531 9585 9603-9604 9729- 9731 9763-9767 9799-9800 9808-9812 9829-9832 9929- 9935 9958-9959 9969-9980 9989-9992 9997-10009 10015-10016 10033-10037 10449-10453 10477-10478 10483 10513-10518 10523-10530 10537-10538 10603- 10608 10638-10639 10780-10782 10901-10902 10931- 10933 10965 11026 11081 11123-11124 11317 11345- 11350 11465-11472 11476-11478 11577 11672 11711- 11712 11731-11734 11739-11740 11803-11804 11934 12102-12110 12117-12118 12131-12132 12202-12208 12215-12217 12226-12228 12333-12334 12374-12377

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induced neuron cells	Stratagene	NTD001	188-191 289-291 576-578 617-618 716-717 1030 1034 1097-1098 1646 2540-2541 2599-2603 2675-2678 2724-2726 2871-2873 2973 3326-3331 3374-3376 3649-3653 3908-3910 3930 3949-3953 4165-4170 4486-4487 4515-4516 4581-4582 4984-4985 5053-5054 5272-5275 5335-5336 5777-5778 5802 5919-5921 5980-5984 6403-6404 6787-6790 6795-6800 7170-7172 7258-7263 7325-7343 7363 7368-7369 7557-7559 7688-7689 7693-7694 7701-7703 7745-7746 7778-7783 7906-7912 7990-7992 8187-8200 8250 8497-8499 8689-8690 8758-8759 8998-9001 9029-9030 9040-9042 9047-9048 9087 9321-9323 9559-9560 9829-9832 9909-9912 9947-9952 9993-9996 10477-10478 10494 10531-10532 10592-10594 10615-10623 10842-10867 10980-10985 11045 11228-11229 11314-11315 11405 11431-11432 11541-11543 11546 11609 11700-11707 11739-11740 11803-11808 11886-11891 11941-11944 12131-12132 12241-12244 12258-12262 12898-12899 12902-12905 12997-12999 13592-13595 13609-13612 13652-13659 14304-14305 15182-15183 15190-15191 15290-15291 15588-15589 15969-15974 16028-16029 16180-16181 16545 16619-16621 16642 17292-17296 17401-17432 17435 17455-17456 18029-18030 18097-18134 18300-18307 18400-18402 18412-18418 18691-18692 18771-18772 18796-18801 18839-18841 18846-18853 18899-18903 18989-18990 19001-19004 19012 19074-19080 19106-19118 19207-19208 19256-19257 19266-19267 19306-19307 19316-19317 19343 19355-19360 19387-19389 19447-19448 19458-19460 19488-19493 19566 19598-19601 19617-19619 19659-19661 19736-19742 19804-19808 19813 19939 19972-19980 20029-20043 20099-20102 20106-20110 20114-20119 20122-20127 20182-20188 20208-20218 20485-20490 20521-20523 20607-20612 20681 20698-20707 20827-20835 20853-20854 20871 21105-21111 21248-21271 21275-21280 21284-21294 21463-21465 21495-21500 21587-21646 21929-21935 22020-22025 22045-22046 22070-22073 22141-22143 22160 22187-22192 22195-22198 22243-22245 22358-22359 22365-22371 22381-22388 22433 22653-22654 22671-22674 22690 22916-22922 22977 23201-23202 23251 23358-23360 23420-23421 23700-23701 23798-23799 23806-23809 23890-23892 24749-24754 24928-24938 24985-24988 25029-25040 25279-25288 25376-25379 26024-26028 26205-26206 26209-26213 26266 26280 26310-26314 26327 26361-26365 26678-26680 27091 27100-27102 27269-27270 27446-27449 27522-27539 27544-27545 27729-27739 27861-27864 27896-27927 27989-27990 28315-28316 28361-28364 29117-29123 29328-29337 29343-29345 29418-29419 29426-29431 29885-29891 30167-30177 30352 30361-30368

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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neuronal cells	Stratagene	NTU001	240-250 373-374 425-427 576-578 847-848 1388-1401 1432-1435 1470 1499 1778-1779 2306 2599-2603 2944 3040-3076 3107-3108 3326-3331 3421-3422 3477-3478 3483-3484 3789-3794 3912-3915 4055-4058 4171-4172 4206-4208 4515-4516 4562-4568 4581-4582 4785-4789 5186-5189 5253-5266 5272-5275 5279-5283 5525-5526 5943-5945 6135-6136 6403-6404 7264-7266 7346-7352 7784-7786 7815-7818 8203 8227-8229 8465 8497-8499 8716-8718 8998-9004 9007-9008 9038-9039 9049-9068 9472-9474 9479-9481 9535-9537 9594-9602 9735-9738 9929-9935 9969-9980 10161-10163 10167-10196 10516-10518 10615-10623 10873-10875 10915-10918 11308-11310 11334-11335 11775-11776 11840 12150-12151 12258 12590-12592 12653-12654 12716-12719 12997-12999 13552-13555 13638-13641 13847-13849 14038-14041 14044-14045 14137-14138 14277-14280 14640-14642 14814-14815 15025-15069 15100-15109 15277-15278 15408-15411 15530-15531 15563-15564 15576-15577 15588-15589 15863-15870 16141-16143 16174-16176 16182-16189 16545 16642 16652 16836-16842 16851-16853 17284-17285 17435-17440 17451-17454 17958-17962 18029-18030 18043 18097-18134 18500-18501 18562-18576 18671-18672 18796-18801 18825-18828 18857-18880 18925-18933 18975 18993-18995 19049-19053 19153 19298-19300 19306-19307 19316-19317 19351-19354 19370 19375-19379 19395-19400 19415-19417 19432-19434 19511 19515-19521 19526-19529 19564-19566 19625 19659-19661 19683-19687 19813-19815 19855-19856 19938 19940-19942 19965-19967 19972-19980 20026-20027 20099-20102 20106-20110 20161-20164 20200-20207 20241 20289 20336-20338 20406-20407 20437-20440 20485-20490 20521-20523 20537-20541 20602-20612 20631-20634 20639-20640 20646-20648 20666-20671 20792-20797 20827-20843 20871 20897-20900 20924-20925 20927-20928 20938-20942 20957-20962 21032-21045 21208-21210 21241-21247 21256-21262 21272-21283 21295-21296 21554-21556 21587-21655 21899-21904 21936-21938 21951-21954 21958-21966 22007-22015 22040-22041 22047-22049 22070-22073 22152-22156 22165-22168 22171-22175 22218-22224 22243-22245 22253-22255 22292-22299 22312-22313 22343-22346 22372-22373

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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pituitary gland	Clontech	PIT004	307-308 395 845-846 1440-1447 1451 1453-1454 2272-2274 2362-2363 3105-3106 3322-3323 3433 4080-4082 4612-4614 4714-4719 4971-4974 5284 5335-5336 5572-5573 6140-6143 6405 6488-6489 7216-7219 7611-7612 7988 8343-8344 8917-8918 9007-9008 9029-9030 9444 9759-9762 10451-10453 10640-10644 10873-10875 11649-11650 11660-11661 11731-11734 11803-11804 11835-11836 12361 12637 13077-13079 13592-13595 14261-14263 14723-14724 15093-15095 15190-15191 15392-15396 16141-16143 16422-16429 16636-16637 16642 16894-16896 18691-18692 18836-18838 18899-18903 18975 19074-19080 19260-19262 19362 19415-19417 19458-19460 19921-19923 20161-20164 20452-20455 20548-20553 20575-20578 20629-20630 20666-20671 21072-21075 21149-21151 21256-21262 21881-21885 21955-21957 22160 22187-22192 22199 22358-22359 22375-22376 22383-22388 22644-22651 22768-22770 22805-22809 22852-22853 22991-22994 23080-23083 23242-23244 23704 23720-23721 23761 26196-26199 26327 26361-26365 26431 26755-26756 27067-27070 27254-27260 27755-27756 27825-27826 27861-27864 28311-28313 29332-29337 29360-29362 29370 30085-30087 30141-30142 30150-30156
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Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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rectum	Invitroge n	REC001	180-181 534-535 540-542 1681-1687 1705-1714 1721- 1722 1745-1746 2272-2274 2675-2678 2695-2698 2813- 2846 3326-3331 3555-3558 3687-3691 3717-3720 3834- 3835 3949-3953 4692-4695 4857-4861 5246 5337-5341 5572-5573 5802-5804 5919-5921 6209-6211 6400-6404 6406-6410 7209-7212 7426-7442 7609 7747-7751 7757- 7759 7778-7783 8074-8078 8323-8325 8329-8334 8453- 8454 8741-8750 8986-8991 9029-9030 9043-9045 9305 9375-9376 9391-9392 9456-9458 9531 9585 9828 9921- 9922 9986-9988 10263-10264 10277 10306 10480-10482

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salivary gland	Clontech	SAL001	260 307-308 331 551-552 832-836 969-971 981-985 1312-1313 1721-1722 1810-1811 2072 2303 2306-2307 2599-2603 2847 2850-2860 3151-3154 3657 3723-3728 3737 3840-3852 3949-3953 4515-4516 4531-4533 4555- 4556 4581-4582 4857-4861 4971-4974 5269-5271 5525- 5526 5652-5653 5658 5700 6337-6338 6411-6412 6442- 6449 6762-6764 7452-7460 7678-7687 7701-7703 7745- 7746 7778-7783 7805 7988 8145-8151 8187-8200 8337- 8342 8383-8389 8554-8555 8986-8991 9018-9020 9038- 9039 9427-9428 9531 9535-9537 9782-9783 9828 9899- 9901 9923-9924 9997-10009 10306 10531-10532 10607- 10611 10876 11009 11123-11124 11609 11644-11648 11669-11671 11731-11734 11835-11836 12040-12041 12175-12176 12202-12205 12229-12230 12362 12434 12468-12469 12474 12565-12568 12573-12574 12642-

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Salivary gland	Clontech	SALs03	981-985 8698-8700 11538-11540 14546-14549 20316 27814-27815 27989-27990 28424
skin fibroblast	ATCC	SFB001	1307 3374-3376 6285-6288 6791-6794 10306 12258 17026-17028 18029-18030 19011 19939 19972-19980 20182-20188 22141-22143 22160 22495 23415-23418 28424 30150-30156
skin fibroblast	ATCC	SFB002	2926 5805-5807 6166-6168 10306 12258 17026-17028 17038-17041 17455-17456 18029-18030 19011 19548- 19553 19813 21060-21061 22141-22143 22160 22373 22495 22531-22533 26879 27636-27639 28424 30150- 30156
skin fibroblast	ATCC	SFB003	5803-5804 6166-6168 12258 17335-17339 18029-18030 18778-18780 19062-19065 19548-19553 20182-20188 22007-22015 23051-23052 23419 25340-25341 27269- 27270 27814-27815 28424 30150-30156
small intestine	Clontech	SIN001	83 87-94 195-200 307-308 332 373-374 557-559 674-675 783-784 852-855 901-904 1071 1240-1241 1470 1678- 1680 1755-1762 1764-1766 1769-1772 2030 2048 2089-

Tissue origin	RNA Source	Library Name	SEQ ID NOS:
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			27600-27601 27814-27815 27819-27820 28142-28145 28233 29367 30141-30142
uterus	Clontech	UTR001	124-125 188-191 227-230 251-252 301 329-331 738-740 919-922 1028 1047 1453-1454 1562-1564 1705-1714 1893-1901 1912-1913 2366 2374-2377 2926 2988-2989 3001 3205-3207 4557-4560 4712-4713 4971-4974 5903- 5906 5919-5921 6114-6136 6235-6237 6403-6404 6533- 6535 6625-6626 6932-6938 7678-7680 7728 7771 7798- 7801 7921-7923 7946 8010-8012 8084-8085 8090-8093 8137-8141 8313-8314 8368-8370 8415 8420-8421 8689- 8690 9072-9074 9264-9265 9517-9521 9535-9537 9577- 9578 9828-9832 9848-9850 9929-9935 9953 10033- 10037 10268-10272 10508-10512 10537-10538 10980- 10985 11071-11075 11135 11505-11506 11546 11609 11731-11734 11803-11804 12023-12026 12046-12049 12190-12191 12378-12397 12432-12433 12894-12897 13107-13117 13592-13597 13888-13895 13954-13956 14058-14059 14261-14263 14445-14447 14604 14650- 14651 14988-14992 15182-15183 15187-15189 15290- 15291 15390 15576-15577 15699-15700 15855-15857 16145-16146 16174-16176 16600 16643-16648 16716- 16723 16851-16853 17330-17332 17454 17958-17962 18015-18016 18527 18655-18658 18673-18677 18761 18789 18825-18834 18894-18896 18899-18903 18936- 18939 19036-19039 19074-19083 19362 19370 19375- 19379 19387-19389 19442-19444 19560-19562 19609- 19615 19693 19727-19732 19764-19767 19816-19818 19926-19929 19933-19937 19950 19981 20029-20043 20120 20122-20127 20146-20148 20151-20154 20289- 20296 20298 20328-20330 20366-20368 20401-20405 20427-20431 20469-20471 20491-20494 20554-20557 20602-20606 20629-20630 20649-20652 20689-20692 20753 20758-20767 20801-20805 20858-20862 20864 20938-20942 21005-21008 21072-21075 21213-21215 21281-21294 21377-21397 21911-21912 21955-21957 21978-21982 22019-22025 22050-22055 22090-22091 22187-22192 22218-22224 22251-22252 22261-22264 22358-22359 22362-22364 22373 22405-22408 22571- 22581 22622-22624 22644-22651 22663-22664 22887- 22891 22955-22957 22969-22970 23047-23050 23094- 23097 23141 23425-23427 23439-23447 23543-23544 24029-24033 24130-24144 25085-25090 25340-25341 25374-25375 25416-25417 26221-26223 26270-26272 26285-26290 26327 26607-26609 26676-26677 26755- 26756 26853-26854 26860-26862 27173-27174 27294 27348-27353 27493-27496 27602-27606 27636-27639 27649-27654 27729-27739 27861-27864 27896-27927 28105-28121 28133-28137 28311-28313 28424 28426- 28428 29339-29340 29378-29379 29962-29963 30150- 30156

*The 16 tissue-mRNAs and their vendor source, are as follows: 1) Normal adult brain mRNA (Invitrogen), 2) normal adult kidney mRNA (Invitrogen), 3) normal adult liver mRNA (Invitrogen),

- 4) normal fetal brain mRNA (Invitrogen), 5) normal fetal kidney mRNA (Invitrogen), 6) normal fetal liver mRNA (Invitrogen), 7) normal fetal skin mRNA (Invitrogen), 8) human adrenal gland mRNA (Clontech), 9) human bone marrow mRNA (Clontech), 10) human leukemia lymphablastic mRNA (Clontech), 11) human thymus mRNA (Clontech), 12) human lymph node mRNA (Clontech), 13) human spinal cord mRNA (Clontech), 14) human thyroid mRNA (Clontech), 15) human esophagus mRNA (BioChain), 16) human conceptional umbilical cord mRNA (BioChain).

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
1	30369	C	1	23	76	
2	30370	B	2	1	735	
3	30371	B	3	1	783	
4	30372	B	4	104	266	
5	30373	B	5	1	1113	
6	30374	C	6	3	164	
7	30375	B	7	112	279	
8	30376	B	8	198	405	
9	30377	B	9	1	687	
10	30378	C	10	346	598	
11	30379	B	11	1	960	
12	30380	B	12	44	350	
13	30381	B	13	264	465	
14	30382	B	14	483	1556	
15	30383	B	15	140	838	
16	30384	B	16	1	372	
17	30385	B	17	1	1404	
18	30386	B	18	25	2013	
19	30387	C	19	1	381	
20	30388	C	20	605	755	
21	30389	B	21	1	912	
22	30390	C	22	124	315	
23	30391	C	23	44	310	
24	30392	B	24	1	330	
25	30393	B	25	1	411	
26	30394	B	26	147	257	
27	30395	B	27	1	597	
28	30396	B	28	201	862	
29	30397	C	29	249	515	
30	30398	B	30	41	816	
31	30399	C	31	26	142	
32	30400	B	32	259	2328	
33	30401	B	33	1	759	
34	30402	B	34	964	2121	
35	30403	C	35	298	449	
36	30404	C	36	115	396	
37	30405	C	37	148	318	
38	30406	C	38	383	483	
39	30407	B	39	1	1125	
40	30408	B	40	1	831	
41	30409	C	41	363	602	
42	30410	B	42	1	324	
43	30411	B	43	64	199	
44	30412	B	44	1	1007	
45	30413	C	45	380	583	
46	30414	B	46	1	432	
47	30415	C	47	1	249	
48	30416	B	48	1	798	
49	30417	B	49	14	1070	
50	30418	C	50	1	225	
51	30419	B	51	1	2673	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
52	30420	B	52	1	258	
53	30421	B	54	1	624	
54	30422	C	55	166	333	
55	30423	B	56	298	380	
56	30424	C	57	139	379	
57	30425	B	58	1	157	
58	30426	B	59	1	447	
59	30427	B	60	1	579	
60	30428	B	61	1	1059	
61	30429	B	62	1	816	
62	30430	B	63	1	558	
63	30431	B	64	1	540	
64	30432	B	65	1	555	
65	30433	B	66	1	648	
66	30434	B	67	1	798	
67	30435	B	68	1	1455	
68	30436	B	69	1	1278	
69	30437	B	70	88	3012	
70	30438	B	71	1	1092	
71	30439	B	72	575	1033	
72	30440	B	73	644	926	
73	30441	B	74	1	1239	
74	30442	B	75	1	1074	
75	30443	B	76	81	467	
76	30444	C	77	44	286	
77	30445	B	78	1	297	
78	30446	B	79	1	978	
79	30447	B	80	72	715	
80	30448	B	81	1	1296	
81	30449	B	82	63	162	
82	30450	C	83	22	420	
83	30451	C	84	201	733	
84	30452	C	85	417	575	
85	30453	B	86	1	267	
86	30454	B	87	112	738	
87	30455	C	88	260	379	
88	30456	B	89	77	399	
89	30457	B	90	158	420	
90	30458	B	91	1	1437	
91	30459	C	92	22	321	
92	30460	B	93	1	843	
93	30461	B	94	142	2798	
94	30462	B	95	887	8434	
95	30463	B	96	1	1014	
96	30464	B	97	1	1197	
97	30465	B	98	16	555	
98	30466	B	99	1	423	
99	30467	B	100	1	651	
100	30468	B	101	233	556	
101	30469	B	102	192	883	
102	30470	C	103	65	274	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
103	30471	C	104	328	546	
104	30472	B	105	80	3900	
105	30473	B	106	1	951	
106	30474	C	107	1	279	
107	30475	C	108	246	368	
108	30476	B	109	1	819	
109	30477	B	110	1	634	
110	30478	B	111	1	379	
111	30479	B	112	80	2747	
112	30480	C	113	139	414	
113	30481	C	114	1	330	
114	30482	B	115	53	618	
115	30483	B	116	1	426	
116	30484	C	117	135	296	
117	30485	C	118	239	432	
118	30486	C	119	381	776	
119	30487	B	120	1	381	
120	30488	C	121	42	175	
121	30489	C	122	1	399	
122	30490	B	123	1	792	
123	30491	B	124	1	894	
124	30492	B	125	1	3498	
125	30493	B	126	8	874	
126	30494	B	127	1	2160	
127	30495	B	128	1	1776	
128	30496	B	129	1	567	
129	30497	B	130	195	728	
130	30498	B	131	1	615	
131	30499	B	132	1	420	
132	30500	B	133	661	2711	
133	30501	B	134	1	621	
134	30502	C	136	1	465	
135	30503	C	137	113	502	
136	30504	C	139	78	269	
137	30505	C	140	98	472	
138	30506	B	141	403	533	
139	30507	C	142	64	315	
140	30508	B	143	1	591	
141	30509	C	144	528	1151	
142	30510	C	145	1	414	
143	30511	B	146	1	936	
144	30512	C	147	91	195	
145	30513	C	148	562	705	
146	30514	C	149	122	313	
147	30515	B	150	566	1535	
148	30516	C	151	75	248	
149	30517	C	152	1	624	
150	30518	C	153	551	655	
151	30519	C	154	315	497	
152	30520	C	155	262	554	
153	30521	C	156	1	282	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, =possible nucleotide insertion)
154	30522	B	157	1	508	
155	30523	C	158	243	545	
156	30524	B	159	8	395	
157	30525	C	160	33	194	
158	30526	B	161	50	355	
159	30527	B	162	128	1230	
160	30528	B	163	243	710	
161	30529	B	164	121	742	
162	30530	B	165	152	227	
163	30531	C	166	156	503	
164	30532	B	167	67	1280	
165	30533	B	168	1	444	
166	30534	B	169	161	206	
167	30535	B	170	189	1207	
168	30536	B	171	1	613	
169	30537	B	172	1	70	
170	30538	C	173	611	751	
171	30539	B	174	398	2472	
172	30540	B	175	87	646	
173	30541	B	176	1	1455	
174	30542	C	177	1	339	
175	30543	B	178	1	1458	
176	30544	B	179	278	766	
177	30545	B	181	85	749	
178	30546	B	182	50	498	
179	30547	C	183	1	522	
180	30548	B	184	90	482	
181	30549	B	185	86	442	
182	30550	C	187	129	308	
183	30551	C	188	1	414	
184	30552	B	190	1	378	
185	30553	C	192	252	308	
186	30554	B	193	1	576	
187	30555	C	194	1093	1311	
188	30556	B	195	45	324	
189	30557	B	196	1	249	
190	30558	C	197	309	443	
191	30559	C	198	615	866	
192	30560	B	199	86	1332	
193	30561	B	200	49	334	
194	30562	B	201	64	638	
195	30563	C	202	195	338	
196	30564	C	203	1	357	
197	30565	B	204	1	693	
198	30566	C	205	121	291	
199	30567	C	206	156	380	
200	30568	C	207	1211	1456	
201	30569	B	208	62	328	
202	30570	C	209	105	179	
203	30571	B	210	229	1483	
204	30572	B	211	1	749	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
205	30573	B	212	1	190	
206	30574	C	213	121	367	
207	30575	B	214	121	456	
208	30576	B	215	1	2631	
209	30577	B	216	63	419	
210	30578	B	217	114	485	
211	30579	B	218	628	1447	
212	30580	C	219	252	377	
213	30581	B	220	1	847	
214	30582	B	221	68	343	
215	30583	B	222	138	911	
216	30584	B	223	44	882	
217	30585	B	224	1	429	
218	30586	B	225	87	312	
219	30587	C	226	44	343	
220	30588	C	227	41	286	
221	30589	C	228	1145	1372	
222	30590	B	229	1	720	
223	30591	C	230	1	430	
224	30592	C	231	58	297	
225	30593	B	232	613	683	
226	30594	B	233	613	683	
227	30595	C	234	238	455	
228	30596	B	235	319	615	
229	30597	C	236	255	494	
230	30598	B	237	106	600	
231	30599	B	238	1	654	
232	30600	B	239	1	654	
233	30601	B	240	243	356	
234	30602	B	241	1	932	
235	30603	C	242	36	215	
236	30604	B	243	1	288	
237	30605	C	244	25	186	
238	30606	B	245	1	574	
239	30607	B	246	1	1257	
240	30608	B	247	162	263	
241	30609	C	248	79	207	
242	30610	B	249	194	276	
243	30611	B	250	1	1671	
244	30612	C	251	118	311	
245	30613	B	252	88	1485	
246	30614	B	253	339	443	
247	30615	B	254	667	1165	
248	30616	B	255	1	981	
249	30617	B	256	450	3131	
250	30618	B	257	900	1199	
251	30619	C	258	5	271	
252	30620	B	259	65	689	
253	30621	C	260	1	321	
254	30622	B	261	1	137	
255	30623	B	262	34	282	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
256	30624	B	263	46	856	
257	30625	C	264	157	468	
258	30626	B	265	148	403	
259	30627	C	266	248	481	
260	30628	B	267	171	393	
261	30629	B	268	1	1078	
262	30630	B	269	1	550	
263	30631	B	270	1	1455	
264	30632	B	271	171	602	
265	30633	B	272	1	1056	
266	30634	B	273	1	1101	
267	30635	B	274	1	2335	
268	30636	B	275	303	419	
269	30637	B	276	1	615	
270	30638	B	277	1	543	
271	30639	B	278	1	1602	
272	30640	C	279	585	1001	
273	30641	C	280	260	379	
274	30642	B	281	1	1437	
275	30643	C	282	22	321	
276	30644	B	283	1	843	
277	30645	B	284	142	2796	
278	30646	B	285	458	7217	
279	30647	B	286	84	186	
280	30648	C	287	67	229	
281	30649	C	288	15	245	
282	30650	C	289	125	232	
283	30651	B	290	1	594	
284	30652	B	291	376	670	
285	30653	C	292	82	405	
286	30654	B	293	35	651	
287	30655	B	294	56	487	
288	30656	C	295	313	498	
289	30657	C	296	118	261	
290	30658	B	297	198	1868	
291	30659	B	298	1	1665	
292	30660	C	299	73	108	
293	30661	B	300	1	408	
294	30662	B	301	1	444	
295	30663	B	302	8	311	
296	30664	C	303	144	350	
297	30665	B	304	1	669	
298	30666	C	305	416	820	
299	30667	B	306	253	837	
300	30668	B	307	44	475	
301	30669	B	308	185	885	
302	30670	C	309	206	337	
303	30671	B	310	1	393	
304	30672	B	311	1	1259	
305	30673	B	312	24	434	
306	30674	B	313	44	2687	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
307	30675	B	314	1	154	
308	30676	B	315	288	770	
309	30677	B	316	85	683	
310	30678	B	317	1	873	
311	30679	B	318	1	1737	
312	30680	C	319	1	690	
313	30681	B	320	58	1487	
314	30682	B	321	1	816	
315	30683	B	322	25	772	
316	30684	B	323	42	271	
317	30685	C	324	16	159	
318	30686	C	325	74	280	
319	30687	C	326	221	545	
320	30688	B	327	192	364	
321	30689	C	328	390	638	
322	30690	B	329	151	4215	
323	30691	B	330	1	2076	
324	30692	B	331	1	465	
325	30693	B	332	40	1350	
326	30694	B	333	1	489	
327	30695	B	334	285	744	
328	30696	C	335	96	347	
329	30697	C	336	213	326	
330	30698	B	337	776	4384	
331	30699	B	338	201	317	
332	30700	B	339	1	2713	
333	30701	B	340	1	894	
334	30702	B	341	1	3842	
335	30703	C	342	745	1131	
336	30704	B	343	82	411	
337	30705	B	344	126	2123	
338	30706	B	345	57	1641	
339	30707	C	346	211	654	
340	30708	B	347	44	266	
341	30709	B	348	1	927	
342	30710	C	349	20	124	
343	30711	C	350	9	455	
344	30712	C	351	188	304	
345	30713	C	352	1	333	
346	30714	C	353	140	298	
347	30715	B	354	73	2171	
348	30716	B	355	1	1374	
349	30717	B	356	150	398	
350	30718	B	357	1	585	
351	30719	B	358	1	1716	
352	30720	B	359	81	1912	
353	30721	B	360	249	770	
354	30722	B	361	474	2875	
355	30723	C	362	1	483	
356	30724	C	363	1	251	
357	30725	C	364	28	407	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
358	30726	C	365	88	204	
359	30727	B	366	474	684	
360	30728	C	367	41	394	
361	30729	B	368	253	1044	
362	30730	B	369	468	1111	
363	30731	B	370	1	558	
364	30732	B	371	21	345	
365	30733	B	372	1	744	
366	30734	B	373	1	795	
367	30735	B	374	1	685	
368	30736	B	375	94	414	
369	30737	C	376	86	268	
370	30738	B	377	1	1003	
371	30739	B	378	41	1385	
372	30740	B	379	1	510	
373	30741	B	380	40	746	
374	30742	B	381	100	1991	
375	30743	B	382	1	267	
376	30744	C	383	168	278	
377	30745	C	384	173	208	
378	30746	B	385	141	4538	
379	30747	B	386	1	4086	
380	30748	C	387	398	474	
381	30749	B	388	1	762	
382	30750	B	389	1	1584	
383	30751	B	390	1	2703	
384	30752	B	391	1	489	
385	30753	B	392	527	780	
386	30754	B	393	1	4050	
387	30755	B	394	859	2958	
388	30756	B	395	639	2307	
389	30757	B	396	1	642	
390	30758	B	397	1	3639	
391	30759	B	398	219	540	
392	30760	B	399	1	3225	
393	30761	B	400	1	7552	
394	30762	C	401	626	1201	
395	30763	C	402	627	827	
396	30764	C	403	1	243	
397	30765	B	404	335	538	
398	30766	B	405	41	409	
399	30767	B	406	160	540	
400	30768	B	407	1	597	
401	30769	B	408	1	1605	
402	30770	B	409	1	351	
403	30771	B	410	65	601	
404	30772	B	411	1	870	
405	30773	B	412	91	2867	
406	30774	B	413	33	410	
407	30775	B	414	298	343	
408	30776	B	415	70	310	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
409	30777	B	416	64	1929	
410	30778	B	417	1	298	
411	30779	B	418	37	2612	
412	30780	B	419	1	510	
413	30781	B	420	44	1111	
414	30782	B	421	26	175	
415	30783	C	422	7	57	
416	30784	C	423	27	230	
417	30785	C	424	7	144	
418	30786	B	425	1	1746	
419	30787	C	426	318	486	
420	30788	B	427	896	1115	
421	30789	C	428	106	309	
422	30790	C	429	52	402	
423	30791	B	430	1	309	
424	30792	B	431	167	492	
425	30793	C	432	144	296	
426	30794	B	433	1	786	
427	30795	B	434	336	1303	
428	30796	B	435	333	419	
429	30797	B	436	1	489	
430	30798	C	437	1	199	
431	30799	C	438	110	239	
432	30800	C	439	175	303	
433	30801	C	440	35	181	
434	30802	B	441	1	1896	
435	30803	C	442	1	331	
436	30804	C	443	71	344	
437	30805	C	444	25	135	
438	30806	C	445	406	595	
439	30807	C	446	148	228	
440	30808	C	447	80	106	
441	30809	C	448	7	375	
442	30810	C	449	300	437	
443	30811	C	450	1	357	
444	30812	B	451	1	729	
445	30813	B	452	58	1287	
446	30814	C	453	1	410	
447	30815	C	454	1	411	
448	30816	C	455	1	420	
449	30817	B	456	1	555	
450	30818	B	457	376	1035	
451	30819	B	458	678	807	
452	30820	B	459	88	1485	
453	30821	B	460	300	2082	
454	30822	B	461	1	819	
455	30823	B	462	780	998	
456	30824	B	463	1	1871	
457	30825	B	464	1	1703	
458	30826	B	465	1	594	
459	30827	C	466	120	245	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
460	30828	C	467	1	387	
461	30829	B	468	1	1678	
462	30830	B	469	1	533	
463	30831	B	470	347	656	
464	30832	B	471	1	1098	
465	30833	B	472	224	1518	
466	30834	C	473	44	244	
467	30835	B	474	1	1251	
468	30836	B	475	1	428	
469	30837	B	476	1	495	
470	30838	C	477	233	373	
471	30839	B	478	8	950	
472	30840	C	479	1	813	
473	30841	B	480	1	1071	
474	30842	C	481	224	418	
475	30843	B	482	39	851	
476	30844	B	483	1	2006	
477	30845	B	484	1	561	
478	30846	B	485	167	227	
479	30847	B	486	1	777	
480	30848	B	487	1	645	
481	30849	B	488	1	1749	
482	30850	C	489	26	847	
483	30851	C	490	243	392	
484	30852	C	491	303	407	
485	30853	C	492	23	300	
486	30854	B	493	131	336	
487	30855	C	494	64	156	
488	30856	B	495	180	712	
489	30857	B	496	1	1104	
490	30858	B	497	24	917	
491	30859	B	498	65	228	
492	30860	B	499	1	2172	
493	30861	B	500	1	1338	
494	30862	B	501	1	795	
495	30863	C	502	181	410	
496	30864	B	503	69	1322	
497	30865	B	504	531	1315	
498	30866	C	505	24	320	
499	30867	B	506	1	791	
500	30868	B	507	1	3256	
501	30869	C	508	361	549	
502	30870	B	509	729	3252	
503	30871	B	510	424	1710	
504	30872	C	511	247	750	
505	30873	B	512	11	124	
506	30874	B	514	116	1079	
507	30875	B	515	1	766	
508	30876	B	516	185	796	
509	30877	B	517	1	456	
510	30878	B	518	99	435	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
511	30879	B	519	1	834	
512	30880	B	520	54	246	
513	30881	B	521	1	372	
514	30882	C	522	78	305	
515	30883	C	523	329	484	
516	30884	B	524	1	459	
517	30885	B	525	630	889	
518	30886	B	526	95	343	
519	30887	B	527	353	610	
520	30888	B	528	113	529	
521	30889	B	529	362	1400	
522	30890	B	530	1	441	
523	30891	C	531	1	327	
524	30892	B	532	1	909	
525	30893	B	534	669	1268	
526	30894	B	535	293	826	
527	30895	C	536	12	155	
528	30896	C	537	1488	1706	
529	30897	C	538	26	211	
530	30898	C	539	30	185	
531	30899	B	540	1	789	
532	30900	B	541	63	358	
533	30901	B	542	1	900	
534	30902	B	543	1	728	
535	30903	B	544	112	220	
536	30904	B	545	49	386	
537	30905	B	546	1	585	
538	30906	B	547	328	531	
539	30907	B	548	10	987	
540	30908	B	549	49	248	
541	30909	B	550	131	368	
542	30910	B	551	80	1098	
543	30911	B	552	1	1364	
544	30912	B	553	1	1294	
545	30913	B	554	1	1995	
546	30914	B	555	1	279	
547	30915	B	556	175	715	
548	30916	B	557	1	636	
549	30917	B	558	1331	1600	
550	30918	B	559	32	406	
551	30919	B	560	38	206	
552	30920	B	561	1	1266	
553	30921	C	562	359	501	
554	30922	B	563	315	465	
555	30923	B	564	94	1683	
556	30924	B	565	1	1570	
557	30925	B	566	139	1734	
558	30926	B	567	1	810	
559	30927	B	568	658	1548	
560	30928	B	569	9	395	
561	30929	B	570	1	567	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
562	30930	B	571	1	567	
563	30931	B	572	1	789	
564	30932	B	573	49	3187	
565	30933	B	574	1	1824	
566	30934	B	575	49	1413	
567	30935	B	576	1	1572	
568	30936	C	577	372	468	
569	30937	C	578	58	225	
570	30938	B	579	79	299	
571	30939	B	580	1	645	
572	30940	C	581	582	749	
573	30941	B	582	170	463	
574	30942	B	583	311	520	
575	30943	B	584	1	1074	
576	30944	B	585	39	140	
577	30945	B	586	60	1685	
578	30946	B	587	106	879	
579	30947	C	588	67	362	
580	30948	B	589	45	126	
581	30949	C	590	1	390	
582	30950	C	591	49	240	
583	30951	B	592	1	496	
584	30952	B	593	94	482	
585	30953	C	594	12	341	
586	30954	B	595	1	354	
587	30955	B	596	1	711	
588	30956	B	597	123	412	
589	30957	B	598	1	1107	
590	30958	B	599	1	800	
591	30959	C	600	82	408	
592	30960	B	601	1	3174	
593	30961	B	602	1	444	
594	30962	B	603	1	1671	
595	30963	B	604	1	603	
596	30964	B	605	339	443	
597	30965	C	606	237	380	
598	30966	B	607	1	771	
599	30967	B	608	1	1767	
600	30968	C	609	1	801	
601	30969	B	610	1	1062	
602	30970	B	611	450	3131	
603	30971	C	612	178	435	
604	30972	C	613	164	319	
605	30973	C	614	1	385	
606	30974	C	615	392	853	
607	30975	C	616	24	200	
608	30976	C	617	34	327	
609	30977	B	618	1	624	
610	30978	B	619	179	1222	
611	30979	B	620	1	916	
612	30980	B	621	151	339	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
613	30981	B	622	135	218	
614	30982	B	623	126	300	
615	30983	C	624	258	467	
616	30984	B	625	58	1038	
617	30985	B	626	246	4677	
618	30986	B	627	1	583	
619	30987	C	628	65	283	
620	30988	B	629	162	909	
621	30989	B	630	1	1062	
622	30990	B	631	1	909	
623	30991	C	632	160	297	
624	30992	B	633	352	1143	
625	30993	C	634	301	459	
626	30994	B	635	1	906	
627	30995	B	636	1	654	
628	30996	B	637	1	528	
629	30997	B	638	1	1102	
630	30998	C	639	81	299	
631	30999	B	640	1	345	
632	31000	B	641	39	360	
633	31001	B	642	22	293	
634	31002	C	643	1	504	
635	31003	B	644	107	3786	
636	31004	B	645	1	576	
637	31005	B	646	66	152	
638	31006	B	647	226	522	
639	31007	B	648	1	49	
640	31008	C	649	50	172	
641	31009	C	650	1	516	
642	31010	B	651	1	615	
643	31011	B	652	1	495	
644	31012	B	653	1	663	
645	31013	B	654	1	1812	
646	31014	B	655	1	1401	
647	31015	B	656	102	1151	
648	31016	B	657	1	385	
649	31017	B	658	232	987	
650	31018	B	659	1	1221	
651	31019	B	660	296	496	
652	31020	B	661	57	285	
653	31021	C	662	203	271	
654	31022	B	663	1	711	
655	31023	C	664	351	542	
656	31024	C	665	420	695	
657	31025	B	666	1	1860	
658	31026	B	667	71	2167	
659	31027	B	668	6	344	
660	31028	B	669	217	693	
661	31029	C	670	1	417	
662	31030	B	671	1	990	
663	31031	B	672	109	1169	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
664	31032	C	673	40	117	
665	31033	C	674	301	560	
666	31034	B	675	1	396	
667	31035	B	676	483	1033	
668	31036	B	677	673	3407	
669	31037	B	678	4	672	
670	31038	C	679	39	116	
671	31039	B	680	1	459	
672	31040	B	681	19	370	
673	31041	B	682	112	704	
674	31042	C	683	387	578	
675	31043	B	684	175	254	
676	31044	B	685	1	501	
677	31045	B	686	290	389	
678	31046	B	687	1	486	
679	31047	B	688	1	651	
680	31048	B	689	181	401	
681	31049	B	690	117	406	
682	31050	B	691	1	169	
683	31051	B	692	1	1539	
684	31052	B	693	1	475	
685	31053	B	694	1	1575	
686	31054	B	695	1	507	
687	31055	B	696	1	498	
688	31056	C	697	253	492	
689	31057	B	698	1	588	
690	31058	B	699	75	291	
691	31059	B	700	1	1355	
692	31060	B	701	112	259	
693	31061	C	702	492	833	
694	31062	B	703	297	483	
695	31063	B	704	45	471	
696	31064	C	705	175	318	
697	31065	B	706	1	1074	
698	31066	B	707	94	1180	
699	31067	B	708	1	3866	
700	31068	C	709	215	424	
701	31069	B	710	1	499	
702	31070	B	711	210	325	
703	31071	B	712	1	786	
704	31072	B	713	1	777	
705	31073	B	714	174	1804	
706	31074	B	715	17	368	
707	31075	B	716	769	1831	
708	31076	B	717	76	301	
709	31077	B	718	1	825	
710	31078	C	719	1	396	
711	31079	B	720	93	2449	
712	31080	B	721	408	687	
713	31081	B	722	97	662	
714	31082	B	723	169	610	

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715	31083	B	724	1	2511	
716	31084	C	725	104	410	
717	31085	C	726	75	527	
718	31086	C	727	7	263	
719	31087	B	728	40	1725	
720	31088	B	729	290	1671	
721	31089	B	730	46	465	
722	31090	C	731	378	644	
723	31091	B	732	48	2331	
724	31092	B	733	1	738	
725	31093	B	734	1	1051	
726	31094	B	735	1	840	
727	31095	C	736	291	551	
728	31096	B	737	1	1308	
729	31097	B	738	1	291	
730	31098	C	739	1	702	
731	31099	B	740	1	379	
732	31100	B	741	80	2747	
733	31101	B	742	1	1992	
734	31102	B	743	293	1296	
735	31103	C	744	769	1017	
736	31104	C	745	166	294	
737	31105	B	746	928	1483	
738	31106	B	747	247	375	
739	31107	C	748	47	582	
740	31108	B	749	47	388	
741	31109	B	750	53	458	
742	31110	C	751	32	277	
743	31111	B	752	1	1641	
744	31112	C	753	1	483	
745	31113	B	754	1	1518	
746	31114	B	755	1	321	
747	31115	C	756	604	779	
748	31116	B	757	695	967	
749	31117	B	758	1	768	
750	31118	B	759	101	531	
751	31119	B	760	1	1014	
752	31120	C	761	424	564	
753	31121	B	762	1	333	
754	31122	B	763	15	165	
755	31123	B	764	1	555	
756	31124	B	765	344	476	
757	31125	B	766	1	648	
758	31126	B	767	1	981	
759	31127	C	768	22	162	
760	31128	B	769	1	225	
761	31129	B	770	232	1671	
762	31130	B	771	166	504	
763	31131	B	772	473	1694	
764	31132	C	773	232	414	
765	31133	C	774	374	463	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
766	31134	B	775	1	1128	
767	31135	B	776	337	1284	
768	31136	C	777	25	282	
769	31137	C	778	4	63	
770	31138	C	779	496	1041	
771	31139	C	780	234	365	
772	31140	B	781	1	669	
773	31141	B	782	228	305	
774	31142	B	783	102	755	
775	31143	B	784	1	465	
776	31144	B	785	45	336	
777	31145	C	786	220	366	
778	31146	B	787	332	456	
779	31147	B	788	169	450	
780	31148	B	789	1	1173	
781	31149	B	790	36	355	
782	31150	C	791	354	482	
783	31151	C	792	328	708	
784	31152	B	793	1	829	
785	31153	B	794	14	182	
786	31154	B	795	307	1412	
787	31155	C	796	3	332	
788	31156	B	797	57	704	
789	31157	B	798	1	2406	
790	31158	C	799	1	759	
791	31159	B	800	1	351	
792	31160	B	801	142	272	
793	31161	B	802	34	2951	
794	31162	B	803	92	994	
795	31163	B	804	115	1746	
796	31164	C	805	292	408	
797	31165	B	806	1	880	
798	31166	C	807	156	329	
799	31167	C	808	119	328	
800	31168	C	809	1	492	
801	31169	B	810	1	516	
802	31170	B	811	1	624	
803	31171	B	812	24	1868	
804	31172	C	813	164	208	
805	31173	C	814	91	249	
806	31174	B	815	1	1059	
807	31175	C	816	80	106	
808	31176	C	817	283	408	
809	31177	C	818	1	357	
810	31178	C	819	1	909	
811	31179	B	820	26	71	
812	31180	B	821	1	714	
813	31181	B	822	1	678	
814	31182	B	823	1	675	
815	31183	B	824	24	1046	
816	31184	B	825	1	933	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
817	31185	B	826	1	363	
818	31186	B	827	112	1655	
819	31187	B	828	1	417	
820	31188	B	829	88	1485	
821	31189	C	830	1	411	
822	31190	B	831	114	277	
823	31191	C	832	671	1039	
824	31192	B	833	63	342	
825	31193	B	834	3530	4798	
826	31194	B	835	1	333	
827	31195	B	836	1	831	
828	31196	B	837	1	2514	
829	31197	B	838	98	250	
830	31198	B	839	1	5247	
831	31199	B	840	1	531	
832	31200	B	841	167	466	
833	31201	B	842	160	417	
834	31202	B	843	215	380	
835	31203	B	844	706	1262	
836	31204	B	845	41	368	
837	31205	C	846	252	578	
838	31206	C	847	18	380	
839	31207	C	848	14	349	
840	31208	B	849	1	1176	
841	31209	B	850	244	1174	
842	31210	C	851	27	146	
843	31211	B	852	217	1866	
844	31212	B	853	98	242	
845	31213	B	854	52	2112	
846	31214	B	855	98	242	
847	31215	C	856	237	518	
848	31216	C	857	1	528	
849	31217	C	858	213	365	
850	31218	B	859	86	478	
851	31219	B	860	1	903	
852	31220	B	861	191	539	
853	31221	C	862	283	480	
854	31222	B	863	248	738	
855	31223	B	864	7	1602	
856	31224	B	865	113	375	
857	31225	B	866	50	435	
858	31226	B	867	50	646	
859	31227	B	868	1	2292	
860	31228	B	869	1	2385	
861	31229	B	870	184	852	
862	31230	B	871	1	408	
863	31231	B	872	218	484	
864	31232	B	873	90	588	
865	31233	B	874	445	625	
866	31234	B	875	138	618	
867	31235	B	876	1	753	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Met hod	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
868	31236	B	877	1	489	
869	31237	B	878	113	366	
870	31238	C	879	271	489	
871	31239	B	880	918	3257	
872	31240	B	881	185	631	
873	31241	C	882	3	194	
874	31242	B	883	80	3219	
875	31243	B	884	213	1835	
876	31244	C	885	132	224	
877	31245	B	886	1	741	
878	31246	C	887	132	224	
879	31247	B	888	1	1281	
880	31248	B	889	125	1910	
881	31249	B	890	1	1449	
882	31250	B	891	284	696	
883	31251	B	892	139	390	
884	31252	B	893	1	1308	
885	31253	B	894	1	594	
886	31254	B	895	1	678	
887	31255	B	896	19	240	
888	31256	B	897	47	330	
889	31257	B	898	1	388	
890	31258	B	899	52	564	
891	31259	C	900	310	672	
892	31260	B	901	1	1338	
893	31261	C	902	77	214	
894	31262	C	903	213	467	
895	31263	C	904	202	426	
896	31264	B	905	68	567	
897	31265	C	906	32	205	
898	31266	C	907	513	701	
899	31267	B	908	1	1083	
900	31268	B	909	787	1633	
901	31269	C	910	40	288	
902	31270	B	911	178	330	
903	31271	B	912	129	520	
904	31272	B	913	2267	2626	
905	31273	C	914	34	87	
906	31274	B	915	23	610	
907	31275	B	916	1	1011	
908	31276	B	917	1	156	
909	31277	B	918	1	754	
910	31278	B	919	1	679	
911	31279	B	920	149	761	
912	31280	B	921	38	1175	
913	31281	C	922	542	724	
914	31282	B	923	31	283	
915	31283	B	924	21	341	
916	31284	B	925	199	361	
917	31285	B	926	293	427	
918	31286	B	927	56	145	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
919	31287	B	928	21	341	
920	31288	B	929	199	361	
921	31289	B	930	293	427	
922	31290	B	931	305	465	
923	31291	B	932	280	457	
924	31292	C	933	45	562	
925	31293	B	934	130	618	
926	31294	B	935	418	1620	
927	31295	B	936	115	252	
928	31296	B	937	1	573	
929	31297	B	938	1	2661	
930	31298	B	939	1	1345	
931	31299	C	940	747	1220	
932	31300	C	941	249	429	
933	31301	B	942	1	363	
934	31302	C	943	390	589	
935	31303	B	944	437	1553	
936	31304	B	945	1	1521	
937	31305	C	946	84	347	
938	31306	B	949	80	315	
939	31307	B	950	1	537	
940	31308	C	951	181	330	
941	31309	C	952	55	123	
942	31310	C	953	52	195	
943	31311	C	954	55	123	
944	31312	B	955	336	648	
945	31313	B	956	1	894	
946	31314	B	957	239	1008	
947	31315	B	958	126	308	
948	31316	B	959	1	747	
949	31317	B	960	101	351	
950	31318	B	961	179	1161	
951	31319	B	962	1	138	
952	31320	B	963	8	791	
953	31321	C	964	218	358	
954	31322	C	965	155	454	
955	31323	C	966	124	303	
956	31324	C	967	1	246	
957	31325	B	968	208	364	
958	31326	C	969	95	256	
959	31327	C	970	312	467	
960	31328	B	971	92	424	
961	31329	B	972	88	147	
962	31330	C	973	434	775	
963	31331	B	974	26	1781	
964	31332	C	975	363	692	
965	31333	B	976	201	563	
966	31334	B	977	348	687	
967	31335	C	978	529	660	
968	31336	C	979	418	738	
969	31337	C	980	25	177	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
970	31338	B	981	308	388	
971	31339	C	982	230	580	
972	31340	B	983	101	342	
973	31341	B	984	1	2341	
974	31342	C	985	1	642	
975	31343	B	986	1	1173	
976	31344	B	987	39	6743	
977	31345	B	988	1	516	
978	31346	B	989	1	756	
979	31347	B	990	1	912	
980	31348	B	991	310	441	
981	31349	C	992	58	300	
982	31350	B	993	80	1344	
983	31351	C	994	325	414	
984	31352	B	995	80	1582	
985	31353	C	996	143	499	
986	31354	B	997	173	375	
987	31355	C	998	126	268	
988	31356	B	999	1	762	
989	31357	B	1000	1	642	
990	31358	B	1001	1	1980	
991	31359	B	1002	67	456	
992	31360	B	1003	48	335	
993	31361	B	1004	1	1251	
994	31362	B	1005	1	642	
995	31363	B	1006	1	570	
996	31364	C	1007	1	687	
997	31365	B	1008	1	5450	
998	31366	B	1009	586	852	
999	31367	B	1010	299	530	
1000	31368	B	1011	1	1659	
1001	31369	B	1012	2	550	
1002	31370	C	1013	2	97	
1003	31371	B	1014	1114	1476	
1004	31372	B	1015	22	822	
1005	31373	C	1016	646	903	
1006	31374	C	1017	1	351	
1007	31375	B	1018	226	1284	
1008	31376	B	1019	138	997	
1009	31377	B	1020	341	527	
1010	31378	B	1021	157	1415	
1011	31379	B	1022	55	211	
1012	31380	B	1023	55	211	
1013	31381	C	1024	18	197	
1014	31382	B	1025	1	876	
1015	31383	B	1026	276	487	
1016	31384	B	1027	1	294	
1017	31385	B	1028	273	377	
1018	31386	B	1029	1	936	
1019	31387	B	1030	1	1158	
1020	31388	C	1031	104	283	

SEQ ID NO:	SEQ ID NO: of peptide sequence	Method	SEQ ID NO: in USSN 09/540,217	Nucleotide location of first codon for peptide sequence	Nucleotide location of last codon for last amino acid of peptide sequence	Amino acid sequence (X=Unknown, *=Stop codon, /=possible nucleotide deletion, \=possible nucleotide insertion)
1021	31389	B	1032	1	720	
1022	31390	B	1033	1	219	
1023	31391	B	1034	1	170	
1024	31392	B	1035	300	831	
1025	31393	C	1036	1	456	
1026	31394	B	1037	1	1149	
1027	31395	B	1038	1	627	
1028	31396	B	1039	161	375	
1029	31397	B	1040	1	360	
1030	31398	B	1041	1	549	
1031	31399	B	1042	1	384	
1032	31400	B	1046	1	675	
1033	31401	C	1047	379	675	
1034	31402	B	1048	166	388	
1035	31403	B	1049	26	66	
1036	31404	B	1050	1	897	
1037	31405	B	1051	30	1359	
1038	31406	B	1052	1	990	
1039	31407	B	1053	52	1507	
1040	31408	C	1054	66	290	
1041	31409	B	1055	158	2072	
1042	31410	B	1056	1	654	
1043	31411	B	1057	51	1143	
1044	31412	C	1058	66	290	
1045	31413	B	1059	547	1510	
1046	31414	B	1060	1	1499	
1047	31415	B	1061	1	3347	
1048	31416	C	1062	116	235	
1049	31417	B	1063	1	1185	
1050	31418	C	1064	221	823	
1051	31419	B	1065	235	359	
1052	31420	C	1066	1	360	
1053	31421	B	1067	49	386	
1054	31422	C	1068	63	383	
1055	31423	B	1069	60	213	
1056	31424	B	1070	1	919	
1057	31425	B	1071	294	557	
1058	31426	B	1072	1	486	
1059	31427	B	1073	1	450	
1060	31428	C	1074	28	207	
1061	31429	B	1075	1	585	
1062	31430	B	1076	60	213	
1063	31431	B	1077	18	457	
1064	31432	B	1078	112	177	
1065	31433	C	1079	1	375	
1066	31434	B	1080	39	91	
1067	31435	B	1081	91	237	
1068	31436	B	1082	255	376	
1069	31437	B	1083	18	431	
1070	31438	B	1084	98	552	
1071	31439	B	1085	1679	1964	